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Abstracts

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Localization of endothelin-1 receptor subtypes in normal and remnant rat kidney. *J. Zhuo, R. Dean, L. Wu, D. Alcorn, D. Casley, and F.A.O. Mendelsohn, Department of Medicine, University of Melbourne, Austin Hospital, Heidelberg, Victoria, Australia.* Endothelin-1 (ET-1) has been implicated in the pathogenesis of acute renal failure after ischemia and in the early development of glomerulosclerosis in remnant kidney. However, the subtype of ET-1 receptors involved in these processes remains unknown. The aim of this study was to localize ET-1 receptor subtypes in sham-operated and remnant rat kidney using *in vitro* macro- and electron microscopic autoradiography and subtype-selective ligands. Two groups of male Sprague-Dawley rats (200 g) were anesthetized with sodium pentobarbital and were either sham-operated ($N = 6$) or underwent a 5/6 nephrectomy (5/6-NPX) ($N = 6$). Five days after surgery the rats were sacrificed and the kidneys removed for ET-1 receptor localization. Frozen sections of both groups of kidneys were incubated with ^{125}I-ET-1 (~0.3 μCi/ml) in 20 mM HEPES buffer, pH 7.4, in the absence or presence of 1 μM unlabelled ET-1, the ET_A receptor antagonist, BQ123, or the ET_B receptor analogue, sarafotoxin 6C (S6C). The densities of ET-1 receptor subtypes were quantified by a computerized densitometry. The 5/6 NPX rats had higher serum levels of creatinine (0.12 ± 0.01 mmol/liter vs. 0.05 ± 0.01 mmol/liter) and urea (18.2 ± 0.01 mmol/liter vs. 4.5 ± 0.2 mmol/liter), and tail-cuff systolic blood pressure (187 ± 9 mm Hg vs. 109 ± 7 mm Hg) than the sham-operated rats. A high density of ET-1 receptors occurred in the glomerulus and the inner medulla, and a moderate density of ET-1 receptors was localized in the outer cortex between glomeruli and in the inner stripe of the outer medulla. There was no significant difference in renal ET-1 receptor binding between sham-operated and 5/6 NPX rats. In both groups of rats, S6C potently inhibited up to 95% of ET-1 receptor binding in all anatomical sites, whereas BQ123 had little effect. In normal rat kidney, electron microscopic autoradiography revealed that ET_B receptor binding (silver grains) was localized almost exclusively in the fenestrated endothelial cells of glomerular capillaries in the cortex and in the endothelial cells of peritubular capillaries in the medulla. Interestingly, both ET_A and ET_B receptor binding occurred in the renomedullary interstitial cells in the inner medulla, whereas no binding was observed overlying the renal tubular cells. These results indicate that the endothelial cells of glomerular and peritubular capillaries are the primary target of ET-1, which may act mainly on ET_B receptors to exert its actions on renal hemodynamics and tubular function in both physiological and pathological states.

Action of epidermal growth factor on circulatory homeostasis in the rat. *S.L. Grant, C.B. Gow, and P.A. Phillips, Department of Medicine, University of Melbourne, Austin Hospital, Heidelberg, Victoria, and Department of Agriculture, La Trobe University, Bundoora, Australia.* Epidermal growth factor (EGF) is best known as a mitogen; however, recent studies have demonstrated a diuresis and natriuresis when administered intravenously to sheep. The aim of this study was to determine if the observed diuresis/natriuresis seen in sheep occurred in other species, and whether EGF produced any hemodynamic changes in a genetic model of hypertension. After a three day baseline period, Sprague-Dawley (SD) (~250 g), Wistar-Kyoto (WKY) and Spontaneously Hypertensive (SHR) rats (17 weeks) received continuous intravenous infusion of either isotonic saline or EGF (2.0 μg/kg hr) via osmotic minipumps and jugular cannulas implanted on day 4 for a further 6 days with daily measurement of urine flow rate, osmolality, sodium and potassium excretion, water and food intake, body weight and systolic blood pressure. Mean arterial blood pressure on conscious free moving rats and glomerular filtration rate (TcDTPA method) were measured on day 5, 48 hours after infusion began. Rats were killed on day 10 and trunk blood collected. After the commencement of infusions those rats receiving EGF significantly increased their water intake ($P < 0.05$) and urine output ($P < 0.05$) and reduced osmolality compared to the controls. Mean arterial blood pressure was lower in those SHR that received EGF (154 ± 10 mm Hg) compared to control SHR (204 ± 3 mm Hg), while there was no change in those normotensive rats, SD control (102 ± 7 mm Hg), EGF (107 ± 8 mm Hg), WKY control (123 ± 5 mm Hg), and EGF (114 ± 5 mm Hg). Food intake over the EGF infusion period was not affected, while body weight increased in all groups. Both sodium and potassium excretion/24 hr were unaffected in normotensive rats while in the SHR both sodium and potassium excretion/24 hr were elevated in those rats receiving EGF. Glomerular filtration rates did not differ between those rats receiving EGF and those rats receiving saline while plasma arginine vasopressin and atrial natriuretic factor also remained unchanged. These findings demonstrate that chronic intravenous infusion of EGF caused diuresis in the SD, SHR and WKY rat, while decreasing the mean arterial pressure only in the SHR. These results raise the possibility that alterations in EGF may play a functional role in renal water and salt homeostasis in the rat and blood pressure control in the SHR.

High affinity amylin binding sites in the proximal tubule, sodium/water resorption and hypertension. *M.E. Cooper, P.J. Harris, J.L. Berka, S. Hiranyachattada, and P.J. Wookey, Departments of Medicine, Physiology and Anatomy, University of Melbourne, Heidelberg Repatriation Hospital, Heidelberg West, and Parkville, Australia.* Amylin, a 37-amino acid peptide, is cosecreted with insulin from the pancreatic β-cells. In the normal rat kidney, we have shown that high affinity (<nM range) binding sites for amylin are associated with the outer cortex (1), and that the binding of [125I]-amylin was competitively inhibited by increasing concentrations of analogues, including the peptide antagonist AC413. These characteristics suggest that this binding site shared similar properties with the amylin or C3 site in the rat brain. Previous studies by our group have also shown that low concentrations of amylin injected into rats or human subjects increase plasma renin by greater than two-fold, and that in the former experiments AC413 administered prior to the amylin injection attenuated this stimulation. The purpose of the studies described here is to pinpoint the exact cellular location of amylin binding using the techniques of injection of radioactive tracer into the renal artery of unconscious animals, removal of the kidney, and analysis of the binding location using high resolution light and electron microscopy, and emulsion autoradiography. As a result of the finding that amylin binding was found almost exclusively in the proximal tubules, we studied the function of low concentrations of amylin at this site. Amylin (~10^{-9} M), introduced on the basolateral side of the proximal tubules in micropuncture experiments, stimulated sodium and water resorption by 29%, making it equipotent with angiotensin II or endothelin. This stimulation was inhibited by the amylin antagonist AC187 and the inhibitor of the Na^+/H^+ exchanger, ethyl isopropyl amiloride, demonstrating the direct functional relationship between the amylin receptor on proximal tubular epithelium and sodium/water resorption. From these results we hypothesize that amylin plays a role in the genesis of hypertension, particularly in those pathophysiological conditions of hyperamylinemia, such as type II diabetes and obesity.

Proteinuria in hypertensive pregnancy: Detection and clinical relevance. *M.A. Brown, M.L. Buddle, and J.A. Whitworth, Departments of Renal Medicine and Medicine, St. George Hospital, Kogarah, NSW, Australia.*

Proteinuria (>300 mg/day) is considered a pre-requisite for the diagnosis of pre-eclampsia and is thought to convey a worse outcome in hypertensive pregnancy. This is often diagnosed by detection of ≥1+ (0.3 g/liter) proteinuria by dipstick testing alone. We conducted studies to determine the accuracy of ward urinalyses for detection of significant proteinuria in hypertensive pregnant women, and to compare the maternal and fetal outcomes of proteinuric and non-proteinuric hypertensive pregnancies. In the first study, ward urinalyses recorded by midwives immediately before and after 285 24 hour urine collections for protein excretion were compared with both total protein excretion and dipstick urinalysis on the mixed 24 hr urine sample by a single observer. Positive predictive values (%) for dipstick testing reflecting true proteinuria are in the Table.

	Nil	Trace	0.3 g/liter (1+)
Pre 24 hour urinalysis	6	16	18
Post-24 hour urinalysis	7	13	23
Aliquot urinalysis	10	15	34

	1 g/liter (2+)	3 g/liter (3+)	>20 g/liter (4+)
Pre 24 hour urinalysis	48	89	83
Post-24 hour urinalysis	64	85	100
Aliquot urinalysis	81	100	100

In the second (prospective) study, maternal and fetal complications were compared between 126 third trimester hypertensive pregnant women with *de novo* proteinuria and 614 without proteinuria, all managed by one physician and a uniform departmental protocol. Maternal complications were all higher in proteinuric pregnancies (neurological 34% vs. 7%; liver disease 21% vs. 8%; thrombocytopenia 20% vs. 8%; renal insufficiency 24% vs. 4%; severe hypertension 57% vs. 13%; all $P < 0.001$). Perinatal mortality was higher in proteinuric pregnancies (48/1000 vs. 5/1000, $P < 0.001$) but fetal growth retardation did not differ significantly (30% vs. 19%, NS). These studies demonstrate that although true proteinuria conveys a worse maternal and fetal prognosis for hypertensive pregnant women, substantial complications still arise without proteinuria, that is non-proteinuric hypertensive pregnancy is not always a benign disorder. Dipstick testing is not sufficiently accurate (both due to observer and dipstick errors) to avoid the need for 24 hour urine collections in these women.

Up-regulation of adhesion molecule expression on endothelial cells (EC) by anti-DNA autoantibodies. *K.N. Lai, K.B. Lai, J. Leung, Department of Medicine, The Chinese University of Hong Kong, Hong Kong.* We examined the effect of polyclonal IgG anti-DNA autoantibodies (dsDNA) purified from 17 patients with systemic lupus erythematosis (SLE) (mean titer 2972 IU/ml) on the expression of intracellular adhesion molecule-1 (ICAM), vascular cell adhesion molecule-1 (VCAM) and E-selectin (ELAM) on human EC using flow cytometry. Compared with IgG from healthy controls [(n = 9), mean dsDNA titer 39 IU/ml], EC incubated with dsDNA expressed a higher mean fluorescence intensity (MFI) in anti-endothelial cell antibodies (AECA) ($P = 0.0001$), von Willebrand factor (vWF) ($P = 0.019$), ICAM ($P = 0.012$), and VCAM ($P = 0.037$), but not in ELAM. dsDNA-depleted polyclonal IgG (dsDNA-dep-IgG) (mean dsDNA titer 23 IU/ml) was prepared from sera of these patients with SLE by affinity chromatography with DNA cellulose column. The MFI of EC incubated with dsDNA was higher than EC incubated with dsDNA-dep-IgG with respect to AECA ($P = 0.005$) vWF ($P = 0.0015$), ICAM ($P = 0.0024$) or VCAM [$P = 0.002$]. Pretreating EC with DNA before incubating with dsDNA did not increase the binding to AECA or expression of vWF, ICAM, or VCAM. The supernatant level of ICAM was significantly elevated in EC cultured with dsDNA as compared with control IgG or dsDNA-dep-IgG. The mRNA encoding for ICAM was similarly raised in EC incubated with dsDNA. Increased proinflammatory activities induced by dsDNA on EC were shown by elevated supernatant levels of interleukin-1β and interleukin-6 in EC incubated with dsDNA. Our findings strongly suggest that anti-DNA autoantibodies play an important pathogenetic role in inducing inflammatory injury of vascular endothelium in SLE.

Comparison of adhesion molecule expression on blood monocytes and peritoneal macrophages from patients on CAPD. *R.J. Faull, J. Wang, and L. Peters, Department of Renal Medicine, and Flow Cytometry Laboratory, Southpath Laboratories, St. George Hospital, Kogarah, NSW, Australia.* Peritoneal macrophages are generally regarded as the first cellular defense against microorganisms invading the peritoneal space. Normally, at least 50% of leukocytes in the peritoneum are macrophages, and during episodes of peritonitis their absolute number increases. The aim of this project is to better understand how peripheral blood monocytes migrate into the peritoneal space, and to study their changing adhesive properties as they differentiate into macrophages. In this study we compared the profiles of adhesion molecules expressed by peripheral blood monocytes and peritoneal macrophages from patients on CAPD. Analysis was by flow cytometry using 22 monoclonal antibodies against different adhesion molecules. The monocyte/macrophage population in blood or peritoneal fluid was determined with a phycoerythrin-labelled anti-CD14 monoclonal antibody. So far we have studied 8 patients on 10 different occasions (1 patient during 2 episodes of peritonitis; another when infection-free after a studied episode of peritonitis). Four episodes were during culture-positive peritonitis (3 *Staphylococcus epidermidis*, 1 *Pseudomonas fluorescens*); 4 culture-negative, clinical peritonitis; 1 infection-free follow-up study; and 1 patient was studied during a dialysis-free recovery period from prolonged renal failure. Several clear patterns have emerged. The following adhesion molecules are consistently down-regulated on peritoneal macrophages compared to blood monocytes: integrins $\alpha4\beta1$, $\alpha6\beta1$, $\alpha L\beta2$ and $\alpha v\beta3$, and CD31 (PECAM-1). Each of these has important roles in monocyte extravasation and migration, but this down-regulation implies that they are less important on tissue macrophages. Only one molecule ($\alpha v\beta5$) is consistently up-regulated on peritoneal macrophages. The exact role of this vitronectin and fibronectin receptor is less well-defined, but this finding suggests that it may be important in macrophage tissue residence. The other adhesion molecules studied either had unchanged expression or exhibited no consistent pattern. Peritonitis had no clear effect on adhesion molecule expression on macrophages or monocytes. These preliminary studies give insights into monocyte/macrophage adhesive behavior and will guide future functional studies.

Up-regulated expression of fibrogenic growth factors is linked to monocyte/macrophage infiltrate in patients with glomerulonephritis. *A.N. Stein-Oakley, G. Perry, N.M. Thomson, Department of Medicine and Renal Unit, Monash Medical School, Alfred Hospital, Melbourne, Australia.* Chronic injury in glomerulonephritis is characterized by glomerulosclerosis, interstitial fibrosis and vascular damage, features consistent with the involvement of fibrogenic growth factors in the pathophysiology of this disease. The expression and distribution of platelet-derived growth factor (PDGF), transforming growth factor β (TGFβ), basic fibroblast growth factor (bFGF), and their receptors, and the severity and nature of interstitial and glomerular leukocyte infiltration were analyzed by immunohistochemistry in biopsy samples from 15 IgA nephropathy (IgAN) patients and 10 focal glomerulosclerosis (FGS) patients. Control renal tissue ($N = 3$) was obtained from areas remote from tumor in nephrectomies performed for renal malignancy. Growth factor expression was up-regulated in varying proportions of IgAN and FGS patients depending on the severity of damage and on the degree of mononuclear cell infiltrate. There were no significant differences in the relative numbers of IgAN vs. FGS patients demonstrating altered growth factor expression. Specific staining patterns were observed for each growth factor. Up-regulation of PDGF AA and PDGF receptors was prevalent in the patient population examined, with lower numbers of patients demonstrating up-regulation of the other growth factors. TGF-β receptors and FGF receptors were ubiquitous in normal and diseased kidneys. The expression of PDGF receptor β (PDGFRβ) was evaluated using an image analysis system, and the numbers of infiltrating leukocytes were counted using an ocular grid. Mean expression of PDGFRβ was increased in IgAN [16 ± 3% in interstitium (I); 28 ± 3% in glomeruli (G)] versus controls (9 ± 3% I; 16 ± 3% G), although differences were not significant due to low control numbers and wide variation between individual patients. There was a strong correlation between interstitial PDGFRβ expression and numbers of $CD14^+$ infiltrating monocytes/macrophages in all patients ($P < 0.01$). Up-regulated glomerular PDGFAA was also accompanied by glomerular $CD14^+$ leucocyte infiltration. The results presented in this study suggest a major involvement of the fibrogenic growth factors in the chronic injury of glomerulonephritis, and demonstrate a strong link between infiltrating

monocytes/macrophages and altered growth factor expression in this pathology.

CD44 expression in rat anti-GBM glomerulonephritis. *J. Zhao, H.Y. Lan, R.C. Atkins, D.J. Nikolic-Paterson, Department of Nephrology, Monash Medical Centre, Clayton, Victoria 3168, Australia.* CD44 is the major cell surface receptor for hyaluronan. Multiple forms of CD44 are expressed through alternative mRNA splicing. The 90 kDa form of the CD44 molecule (termed CD44H) is expressed by cells of hemopoietic and mesodermal origin. A number of functions have been ascribed to CD44H, including lymphocyte homing and extracellular matrix adhesion. We have examined CD44 expression in rat accelerated anti-GBM glomerulonephritis (days 1, 7, 14, 21 and 28) by immunoperoxidase staining of PLP-fixed cryostat sections using the OX-49 mAb which recognizes the 90 kDa form of CD44 expressed by lymphoid cells. In normal kidney, CD44 is expressed by Bowman's capsule parietal epithelium, occasional interstitial cells, medullary tubules, thick ascending limb of Henle and distal tubules. CD44 expression was strongest on the basolateral surface of tubular epithelial cells. In anti-GBM glomerulonephritis strong CD44 expression was seen on infiltrating glomerular neutrophils and macrophages during the first 24 hours following antibody deposition on the GBM. However, at later time points there was little CD44 expression by glomerular macrophages, except that CD44+ cells were prominent in cellular crescents. From day 14 onwards there was a marked increase in the number of CD44+ tubules which was evident in focal areas of tubular damage with prominent local CD44+ leukocytic infiltration. In addition, areas of fibrosis (particularly crescents and periglomerular) also exhibited anti-CD44 staining which did not appear to be cell associated, but may reflect shed CD44 antigen. In conclusion, CD44 expression in rat anti-GBM glomerulonephritis was associated with the early glomerular leukocytic infiltrate, glomerular crescent formation, localization of interstitial leukocytes in areas of focal tubular damage and fibrosis.

Genetic predisposition to TH1 type T helper cell responses favors crescent formation in glomerulonephritis. *X.R. Huang, S.R. Holdsworth, P.G. Tipping, Centre For Inflammatory Disease, Monash University Department of Medicine, Monash Medical Centre, Clayton, 3186, Victoria, Australia.* The Th1 subset of T helper cells is identified by its capacity to produce the cytokines interferon-γ, tumor necrosis factor-β and interleukin-2. Th1 cells are involved in direct T cell-mediated immune responses, typified by delayed type hypersensitivity (DTH). The Th2 subset of T helper cells produces interleukin-4 and interleukin-10 and helps in antibody production. Glomerular crescent formation shows features of classical DTH with local T helper cell and macrophage accumulation and fibrin deposition, suggesting a Th1 type response. To assess the contribution of Th1 and Th2 T cell subsets to the development of glomerular crescent formation, glomerulonephritis (GN) induced by antigen representation in the glomerulus of sensitized mice was compared in strains known to produce predominantly a Th1 (C57BL/6) or Th2 (BALB/c) type immune response to antigens. Anti-glomerular basement membrane (GBM) GN was induced by the i.v. injection of sheep anti-mouse GBM globulin to mice presensitized ten days earlier with sheep globulin in Freund's complete adjuvant. This produced a proliferative GN with proteinuria and renal impairment (increased serum creatinine). Ten days after initiation of GN, C57BL/6 mice showed a more cellular proliferative pattern of GN, with significantly more crescent formation (41 3% of glomeruli with crescents compared to 10 3% of glomeruli in BALB/c mice, $P < 0.01$). Glomerular fibrin deposition assessed by immunofluorescence was more extensive in C57BL/6 mice than that in BALB/c mice. Circulating mouse anti-sheep antibody levels (assessed by ELISA) and deposition of mouse immunoglobulin in glomeruli (assessed by immunofluorescence) were no different in the two strains. Glomerular C3 deposition was less prominent in C57BL/6 mice. These studies demonstrate that a dominant Th1 type T helper cell response favors the development of crescents in GN independent of the humoral immune response. This suggests that Th1 T helper cells contribute to glomerular crescent formation via DTH-like mechanisms.

The proinflammatory and cytotoxic activities of TNF-α. *J.A.J. Barbara, W.B. Smith, X. VanOstade, W. Fiers, M.A. Vadas, and A.F. Lopez, Division of Human Immunology, Hanson Centre for Cancer Research, Frome Rd., Adelaide, South Australia 5000.* Tumor necrosis factor-alpha (TNF-α) is a pleiotropic cytokine which has been administered *in vivo* as an antineoplastic agent and is implicated in the pathophysiology of SLE, transplant rejection, CMV reactivation, hemolytic-uremic syndrome and nephrotic syndrome. However, the molecular mechanisms through which TNF-α expresses its biological activities are only now being unraveled. Using TNF mutants with selective binding to the two TNF receptors (TNFR55 and TNFR75), the receptor biology of TNF was examined in human neutrophils, endothelium and malignant cell lines. The TNFR55-selective mutants resulted in neutrophil and endothelial proinflammatory activities which were reduced up to 170- and 280-fold, respectively, when compared to wild-type TNF. However, these mutants shared similar activity to wild-type TNF in causing cytotoxicity of a human carcinoma-derived cell line and cytostasis in a human leukemic cell line. The TNFR75-selective mutant did not directly mediate any of the proinflammatory activities examined giving an important insight into the mechanism of action of TNF-α. In addition, the TNFR55-selective mutants resulted in neutrophil apoptosis, as determined by microscopy (light and fluorescent) and DNA fragmentation gels, which was significantly decreased compared to wild-type TNF (ED_{50} wt 7.9 ng/ml, E146K 115.7, $P < 0.05$, R32W-S86T 97.6, $P < 0.005$) and indicates that similar mechanisms may be involved in the noninflammatory removal of these cells after functional activation. These results have implications not only for the treatment of malignancies such as renal cell carcinoma, but also for the design of other specific therapeutic modalities.

Tamm-Horsfall protein (THP) in rat anti-GBM glomerulonephritis (GN). II. Is it a leukocyte mitogen? *S. Qing, D.J. Nikolic-Paterson, R.C. Atkins and H.Y. Lan, Department of Nephrology, Monash Medical Centre, Clayton, Victoria 3168, Australia.* THP has been reported to be a leukocyte mitogen *in vitro*. However, in the accompanying abstract we demonstrated the development of an antigen-specific immune response to THP in rat accelerated rat anti-GBM GN. This raised the question of whether THP acts as a conventional antigen or as a mitogen in this disease. We confirmed the mitogenic activity of highly purified rat THP *in vitro*. Splenocytes isolated from normal rats or animals sensitised to THP were cultured for 36 or 72 hrs with THP (50 to 400 μg/ml) and the high level of ^{3}H-thymidine incorporation was equivalent to that obtained with a conventional mitogen, Concanavalin-A (5 μg/ml). Double immunohistochemistry found that 10 to 20% of T cells, B cells and macrophages expressed the proliferating cell nuclear antigen (PCNA). This was abrogated by the addition of anti-THP antibody. Next, we looked for evidence of a mitogenic effect of THP upon leukocytes in rat anti-GBM GN. Leukocytes, PCNA and THP were co-localized using triple immunohistochemistry staining. In normal rat kidney, the resident interstitial leukocytes ($OX\text{-}1^+$) showed no evidence of proliferation. During development of rat anti-GBM GN there was significant accumulation of T cells and macrophages around THP^+ tubules (i.e. contacting the tubule), which was 3- to 4-fold greater than that present around THP^- tubules. Some 0.8 ± 0.2 $OX\text{-}1^+PCNA^+$ cells per THP^+ tubule (distal and thick ascending limb of the loop of Henle) were seen on day 1. This number increased to 2.4 to 2.6 $OX\text{-}1^+PCNA^+$ cells/THP^+ tubule (distal and thick ascending limb of the loop of Henle) were seen on day 1. This number increased to 2.4 to 2.6 $OX\text{-}1^+PCNA^+$ cells/THP^+ tubule from day 7 onwards. This accounted for 27 to 36% of $OX\text{-}1^+$ cells around THP^+ tubules and was comprised of both $R73^+$ T cells and $ED1^+$ macrophages. However, a very similar percentage of $OX\text{-}1^+$ cells around THP^- tubules (22 to 33%) was also $PCNA^+$, and again this was comprised of both T cells and macrophages. In conclusion, we have found that, while THP acts as a mitogen *in vitro*, there is no evidence of such an activity *in vivo*. The differences in THP behavior requires further investigation.

Lack of large deletions affecting 3′ end of locus for autosomal dominant polycystic kidney disease in 73 affected individuals. *J. Ellis and J. Savige, University Department of Medicine, Austin Hospital, Heidelberg, Victoria 3084, Australia.* The abnormal gene in patients with autosomal dominant polycystic kidney disease (ADPKD, PKD1) is located on the short arm of chromosome 16 and encodes a novel protein, PBP (polycystic kidney disease breakpoint protein). A large part of this gene is duplicated nearby on the same chromosome, and this has made it difficult firstly to identify the mutant gene, and secondly to screen the DNA for mutations. We used PCR primers to amplify the unreduplicated 3′ end of the PBP gene from the DNA of 73 individuals with ADPKD. The amplified product is about 400 bp long, and in no individual was a shortened or absent product observed. Initially it was thought that mutations probably took place more

often within this region of the ADPKD gene, but our results suggest that large deletions within the 3′ end of the PBP are rare.

Alpha1-antitrypsin deficiency and anti-proteinase 3 antibodies in ANCA-associated systemic vasculitis. *J.A. Savige, L. Chang, M. Daskalakis and J. Doery, Molecular Medicine, University Department of Medicine, Austin Hospital, Melbourne, Victoria, and Biochemistry Department, Monash Medical Centre, Clayton, Victoria, Australia.* Alpha1-antitrypsin (A1-AT) is a naturally-occurring inhibitor of proteinase 3 and elastase, two of the target antigens of anti-neutrophil cytoplasmic antibodies (ANCA). These antibodies are often associated with Wegener's granulomatosis and microscopic polyarteritis. An increased incidence of A1-AT phenotypes that result in dysfunctional protein or low levels has been reported in patients with anti-proteinase 3 antibodies, and we have looked at this association. Phenotypes associated with a moderate or severe deficiency of A1-AT were found more often in individuals with anti-proteinase 3 antibodies than in the general population: 4 of 31 patients with anti-proteinase 3 antibodies (13%) had the phenotypes MZ ($N = 2$), S ($N = 1$) or Z ($N = 1$), $P < 0.01$). However, overall levels of A1-AT were normal (12/26, 46%) or elevated (14/26, 54%) in patients with anti-proteinase 3 antibodies, including the patients with deficient phenotypes. There were no abnormal phenotypes demonstrated in any sera from patients with anti-elastase, anti-myeloperoxidase or anti-glomerular basement membrane antibodies. ANCA were not demonstrated by indirect immunofluorescence in the serum from any of 73 patients with abnormal A1-AT phenotypes, including 23 individuals with ZZ. We have confirmed an association between abnormal A1-AT phenotypes and the demonstration of anti-proteinase 3 antibodies, but there is no reduction in levels of A1-AT in these individuals. The development of anti-proteinase 3 antibodies may relate to the increased propensity of unbound and uninhibited proteinase 3 to stimulate autoantibody production.

Antibodies to endothelial cells (AECA) and epithelial cells (AEpCA) in IgA nephritis (IgAN) and lupus nephritis (LN). *L.M. Johnstone, R.G. Walker, G.J. Becker, Department of Nephrology, Royal Melbourne Hospital, Parkville, Victoria, 3052, Australia.* AECA have been described in several diseases including LN, IgAN, vasculitis, diabetes, thrombomicroangiopathies and renal transplantation. Their significance remain unclear. Previously, we have shown in LN and IgAN patients increased levels of AECA compared to Controls (C) and that some AECA positive sera from IgAN patients showed binding activity on fibroblasts suggesting that endothelial cells (EC) and fibroblasts may share common antigens recognised by these sera. In this study we have investigated the presence of AECA and antibodies to kidney epithelial cells in sera from patients with IgAN and LN. AECA and AEpCA were detected using a cellular ELISA. AECA were tested against human unfixed umbilical vein EC. AEpCA were tested against monkey kidney epithelial cells (CSL) in a commercial cell line. Binding the cells were exposed with goat anti-human immunoglobulin G (IgG) and goat anti-human total immunoglobulin (Ig) with an alkaline phosphatase label. All sera were tested in triplicate. An ELISA ratio (ER) was calculated for each set of sera, i.e., ER (%) = [(sample OD − negative control OD)/(positive control OD − negative control OD)] × 100.

		IgG %	Total Ig %
AECA	C ($N = 22$)	10.4 (1.4–37.0)	17.1 (5.3–48.2)
	IgAN ($N = 69$)	25.7 (3.9–172.9)[a]	30.3 (3.4–118.4)[b]
	LN ($N = 17$)	15.8 (3.0–157.6)	13.3 (6.1–148.6)
AEpCA	C ($N = 22$)	22.3 (0.0–88.8)	29.0 (0.0–90.6)
	IgAN ($N = 66$)	60.9 (0.0–119.9)[c]	56.3 (0.0–143.0)
	LN ($N = 16$)	30.5 (14.1–81.0)	32.4 (16.3–68.3)

Median (range). [a] $P = 0.0004$, [b] $P = 0.0017$, [c] $P = 0.0002$, Wilcoxon rank-sum test compared to C.

No correlation was evident between AECA and AEpCA, IgG or Total Ig level (Spearmans test, data not shown). We concluded that AECA and AEpCA were present in IgAN but were not demonstrated in LN. Further work is required to determine the nature of antigenic determinants recognised by AECA and AEpCA.

Cyclosporine in renal transplantation: A nine-year experience. *Z.F. Yuan, R.R. Bailey and J. Gardner, Departments of Nephrology and Pathology, Christchurch Hospital, Christchurch, New Zealand.* Cyclosporine A (CsA) has been used in a triple immunosuppressive regimen in our renal transplant program since 1 October 1984. From that time until 30 June 1993, 117 patients had a total of 130 renal transplants. This review was confined to 52 of these patients who had been on CsA for at least 12 months and had remained under regular surveillance in this clinic; 65 were excluded for various reasons (transferred to home town, 27; graft failed within 12 months, 22; follow-up <12 months, 11; miscellaneous, 5). Patients were given 10 mg/kg of CsA prior to transplantation then 8 mg/kg/day in two divided doses after the onset of graft function. The maintenance dose was tapered to a mean of 3.5 mg/kg/day (SD 1.4) within 9 months, and thereafter ranged from 3.0 to 3.4 mg/kg/day. Individual patients differed significantly in the trough whole blood CsA concentrations that they produced on a certain dose. Definite chronic CsA nephrotoxicity occurred in three patients and equivocal changes in another patient, while on a CsA dose of 3.0 to 4.0 mg/kg/day. CsA was an effective drug as part of a triple immunosuppressive regimen for renal transplantation. Chronic CsA nephrotoxicity was an unpredictable finding, and occurred with doses as low as 3.0 to 4.0 mg/kg/day and with trough drug concentrations within the recommended therapeutic range. All patients on CsA should be under regular supervision and early graft biopsy undertaken if graft dysfunction occurs.

Predicting GFR after renal transplantation. *B.J. Nankivell, J.R. Chapman, Department of Renal Medicine, Westmead Hospital, NSW, 2145, Australia.* Serum creatinine is an important marker of the impairment of glomerular filtration rate (GFR) after kidney transplantation. Predictive formulae (such as Cockroft and Gault) derived from patients with chronic renal failure using creatinine clearance are inaccurate when applied to kidney transplant recipients. The purpose of this study was to investigate causes of this inaccuracy and to derive specific predictive GFR formulae appropriate to kidney transplant recipients. Predictive factors for isotopic GFR, serum creatinine and muscle mass were evaluated in consecutive kidney recipients ($N = 146$) using Tc^{99m} DTPA GFR measurements ($N = 751$) as a reference method. Factors which predicted GFR apart from serum creatinine included sex, height, body weight, serum urea, years on dialysis, number of rejection and infective episodes, and prednisolone dose. The relationship between serum creatinine and GFR was highly variable and dependent on factors that alter muscle mass and muscle catabolic rate. Further variation was introduced by clinical situations where tubular secretion of creatinine may be reduced such as ATN and chronic rejection. Three GFR formulae (according to availability of clinical data) were specifically derived and tested against six published methods of GFR estimation: GFR (ml/min) = 6.7/creatinine (mmol/liter) + body wt (kg)/4 − urea(mmol/liter)/2 − 100/height(m)^2 + [35(♂) or 25(♀)]. These derived formulae had the highest correlation, no overall bias, least scatter of sum of squares, and least error at low levels of GFR. They provide a better estimation of GFR in kidney transplantation and their use is recommended when isotopic methods are not available or inappropriate.

The mechanisms of cyclosporine toxicity induced by clarithromycin. *S.T. Spicer, C. Liddle, J. Chapman, P. Barclay, B. Nankivell, P. Thomas, P. O'Connell, Department of Renal Medicine, Westmead Hospital, NSW, Australia.* Recently a number of case reports have described the interaction of clarithromycin with cyclosporine A, resulting in cyclosporine toxicity. Following a case of cyclosporine toxicity with acute renal failure in a clarithromycin-treated transplant patient, the effect of oral clarithromycin on the hepatic P450 system was investigated in five healthy male volunteers using the erythromycin breath test (EBT). Hepatic P450-3A enzymes form the main mechanism for elimination of cyclosporine A, and the EBT is a specific probe for the activity of these enzymes. Each subject underwent an erythromycin breath test in which the amount of radioactive CO_2 exhaled was correlated with the activity of hepatic P450-3A in each volunteer. One week later each volunteer received clarithromycin 500 mg, q12 hourly for 48 hrs (i.e. 4 doses), and the EBT was repeated to determine if hepatic P450-3A had been inhibited. There was a significant reduction in the area under the curve (AUC) of exhaled C^{14} erythromycin. The mean reduction in the AUC was 26.2%. The degree of reduction varied widely from 16.3% to as much as 35.9% ($P = 0.0075$). We conclude

that clarithromycin causes cyclosporine toxicity by inhibiting its metabolism via the Hepatic P450 enzyme system. The wide intersubject variability may explain the variable effect on cyclosporine levels noted clinically, and suggests competitive inhibition rather than complexing of the P450 3A enzymes as the mechanism of this interaction.

Use of acitretin for the skin complications in renal transplant recipients. *Z.F. Yuan, A. Davis, K. Macdonald, and R.R. Bailey, Departments of Nephrology and Dermatology, Christchurch Hospital, Christchurch, New Zealand.* Fifteen long-term Caucasian renal transplant recipients suffering skin complications were treated with acitretin, a second generation retinoid. Indications for its use were progressive actinic keratoses, widespread warts or recurrent skin malignancies. The daily dose ranged from 10 to 50 mg. Therapeutic benefit was assessed by the general condition of the skin and the number of skin malignancies excised. Side effects and toxicity were monitored by regular enquiry, skin examination, liver function tests and lipid profiles. All patients experienced subjective improvement with the skin becoming softer and smoother. Actinic keratoses and warts improved or disappeared. Six patients had been on acitretin for >12 months. The number of malignancies were decreased in 4 of these 6 patients. Nine of the 15 patients had skin side effects, including dry lips, hair thinning, skin scaling, nail changes and photosensitivity. Acitretin had to be reduced in dose in these 9 patients and stopped in 4. There were no biochemical side effects. Seven patients were on cyclosporine and no interaction was found with acitretin. In summary, most of the patients experienced subjective improvement and actinic keratoses and warts were improved or disappeared. The effect on the skin malignancy rate was variable. The benefits of acitretin treatment are still anecdotal and there is a need for a multicentre, prospective, randomized study.

Combined liver-kidney transplantation in the treatment of primary type 1 hyperoxaluria. *P. Branley, P. Angus, R. McL. Jones, R.A. Sinclair, and P.J. Miach, Renal & Liver Units, and Department Anatomical Pathology, Austin Hospital, Heidelberg, Victoria, Australia.* Type 1 hyperoxaluria presents in children or young adults with bone disease or renal disease due to oxalate accumulation from deficiency in the hepatic enzyme alanine, glyoxylate aminotransferase. The clinical severity of disease varies with the amount of residual enzyme. Liver transplantation corrects the enzyme deficiency and combined liver-kidney transplantation is the treatment of choice for patients with renal failure. We report three cases of combined liver-kidney transplantation in this condition. Renal transplantation alone failed in two cases due to recurrent renal oxalosis and renal failure. All patients survive with a functioning liver transplant after 2-1/2, 3-1/2 and 4-1/2 years. However high oxalate excretion has continued in all patients despite good renal function (plasma creatinine 0.12–0.15 mmol/liter) causing loss of one kidney transplant after 3 years. A second kidney has failed due to an undefined cause. Patients with primary oxaluria should be considered for liver or liver kidney transplantation before heavy oxalate deposition occurs in kidney and bone if subsequent loss of kidney function due to oxalosis is to be avoided.

Hepatitis B virus (HBv) associated chronic liver disease in cyclosporine-treated renal allograft recipients. *D. Roy, B. Ramakrishna, B.S. Ramakrishna, C.K. Jacob, J.C.M. Shastry. Departments of Nephrology, Pathology and Gastroenterology, Christian Medical College & Hospital, Vellore, India.* Hepatitis B virus associated chronic liver disease is an important cause of long-term mortality and morbidity in renal allograft recipients. Most studies have been prior to the cyclosporine era. Whether cyclosporine based immunosuppression alters the course or the development of chronic liver disease is not entirely clear. A total of 55 hepatitis B surface antigen (HBSAg) positive recipients of live-related donor renal allografts were studies. They either received azathioprine-prednisolone (AZA) (33 patients) or cyclosporine (CsA) (22 patients) based immunosuppression. The mean follow-up was 37.8 + 17 months and 58.2 + 22.6 months for the AZA and CsA groups, respectively. Coincident infection with hepatitis C was not uncommon (10%). Chronic liver disease developed in 47% of the HBsAg positive renal allograft recipients. All patients with HCV coinfection developed chronic liver disease. Liver biopsies were performed in 15 patients. The biopsies revealed chronic active hepatitis 8, chronic persistent hepatitis 1, cirrhosis 1, granuloma 2, cholestasis 2 and normal 1. Nine patients died, 6 from the AZA group and 3 from the CsA group. Liver cell failure with septicemia was the cause of death in 8 patients. Hepatitis B virus associated chronic liver disease, a major cause of morbidity and mortality after renal transplantation in India, is not significantly different with either of the currently available immunosuppressive regimes.

Mechanism of Na transport inhibition during chronic volume expansion. *A.Z. Györy, N. Salipan-Moore and S. Reddy, Departments of Medicine and Renal Medicine Royal North Shore Hospital and University of Sydney, NSW, Australia.* In acute extracellular fluid volume expansion (AcVE), proximal tubular Na transport inhibition has two components: a 20% inhibition when measured with a fluid resembling proximal tubular fluid (ATF) and an additional 30% inhibition with native harvested proximal tubular fluid (HTF). Because the natriuresis in chronic volume expansion (ChVE) is more modest than in AcVE, different tubular mechanism may be involved in the inhibition of Na transport in these two models. To test this, a ChVE model (4% NaCl diet) was set up in rats to examine proximal tubular Na transport with ATF and HTF Plasma volume was expanded by 13% as assessed by changes in Ht and serum albumin concentration was maintained at normal levels (48.1 ± 1.0 in ChVE and 46.6 ± 3.4 g/liter in controls, $P = 0.46$). Proximal tubular Na transport (Jv, $mm^3 \cdot mm^{-2} \cdot sec^{-1}$) was measured with the shrinking drop technique. With ATF, proximal tubular Na transport was $3.67 \pm 0.09 * 10^{-4}$ ($N = 74$) in ChVE rats and $3.70 \pm 0.26 * 10^{-4}$ ($N = 18$) in controls ($P = 0.89$) and $2.78 \pm 0.07 * 10^{-4}$ ($N = 92$, $P < 0.0001$) with HTF, compared to ATF in the ChVE rats. This reduction of transport to 75.6% agrees closely with that obtained previously with HTF alone in AcVE. Intracellular Na measured with electron-microprobe analysis was increased in ChVE (20.2 ± 0.08 vs. 18.0 ± 0.7, $P = 0.044$), while Cl, P and dry weight decreased significantly, the latter two indicating cell swelling. In conclusion: (1) proximal Na transport was only inhibited with HTF providing evidence that, at least in proximal tubules, with HTF, similar mechanisms are involved in the Na transport inhibition in both types of VE; and (2) that this transport inhibition is accompanied by evidence of Na pump inhibition.

Is the K^+ conductance of the proximal tubule regulated by cytosolic pH? *M. Bleich, M. Hug, A.Z. Györy, and R. Greger, Albert-Ludwigs-Universität, Freiburg, Departments of Medicine University of Sydney, NSW, Australia.* A high K^+ conductance of proximal tubule cells is a prerequisite for luminal Na^+ uptake and basolateral $Na^+HCO_3^-$ cotransport. Patch-clamp and fluorescence measurements were performed on freshly isolated proximal tubules of rat kidney to study if there is a link between intracellular pH and cellular K^+-conductance. Basic properties of freshly isolated proximal tubules: the membrane voltage (V_m) was -57 ± 2 mV ($N = 40$), an increase of extracellular K^+ by 20 mmol/liter depolarized V_m by 19 ± 2 m ($N = 19$), and glucose (10 mmol/liter) depolarized V_m by 12 ± 2 mV ($N = 19$). In fluorescence measurements the effects of changes in extracellular pH or CO_2 and HCO_3^- on intracellular pH were examined (changes in emission ratio (Δratio) are given as a measure of intracellular pH). The effects on V_m and single channel open probability (P_o) were determined in separate experiments. Cells were alkalinized by an increase in extracellular HCO_3^- and CO_2 at constant external pH: Δratio of fluorescence measurements was 13 ± 2% ($N = 11$), membrane potential hyperpolarized: $\Delta V_m = -7 \pm 2$ mV ($N = 30$) and channel open prabability increased: P_o changed from 0.26 to 0.56 ($N = 2$). Intracellular pH was also increased by reduction of CO_2 at constant external HCO_3^-: Δratio = 18 ± 3% ($N = 3$) ΔVm = 8 ± 1 mV ($N = 5$), and by DIDS (10^{-4} mol/liter Δratio = 6 ± 2 ($N = 8$), $\Delta V_m = 41$ mV ($N = 4$). Cells were acidified by an increase in CO_2 at constant HCO_3^-: Δratio = −17 ± 2% ($N = 6$), $\Delta V_m = +12 \pm 2$ mV ($N = 4$), P_o changed from 0.27 ± 0.08 to 0.08 ± 0.03 ($N = 3$). Intracellular pH was also decreased by removal of CO_2 and HCO_3^- at constant pH: Δratio = 20 ± 2 ($N = 4$), $\Delta V_m = +8 \pm 1$ mV ($N = 5$) and P_o changed from 0.23 to 0.11 ($N = 2$). The use of the ammonium pulse technique for variation of intracellular pH could not be applied because NH_4^+/NH_3 itself blocked K^+ channels ($N = 3$) in this preparation. The present data indicate that proximal tubular K^+-conductance is pH-regulated. Membrane potential may serve as a direct feedback signal for basolateral electrogenic $Na^+HCO_3^-$ transport maintaining intracellular pH in a physiological range despite of high HCO_3^- transport rates.

Is one mechanism of Na transport inhibition by volume expansion via the apical Na/H exchanger or basolateral $Na[HCO]_3$ cotransporter? *A. Györy, M. Bleich, M. Hug, and R. Greger, Albert-Ludwigs-Universität, Freiburg, Germany, and Department of Medicine, University of Sydney,*

Australia. In acute sustained extracellular fluid volume expansion (AcVE), the mechanism of proximal tubular (PT) Na transport inhibition has two components: a 20% inhibition measured with a fluid resembling proximal tubular fluid (ATF) and an additional 30% inhibition with harvested proximal tubular fluid (HTF). In transfer experiments we have shown that the former is an expression of a PT epithelial change, whereas the latter is due to a factor contained in PT fluid of AcVE animals. Patch-clamp and fluorescence measurements were performed on freshly isolated proximal tubules of control (Co) and acutely volume expanded (Ex) rat kidneys. Basic properties of freshly isolated proximal tubules: membrane voltage (V_m) was -57 ± 2 mV ($N = 40$), an increase of extracellular K^+ by 20 mmol/liter depolarized V_m by 19 ± 2 mV ($N = 25$), and glucose (10 mmol/liter) depolarized V_m by 15 ± 3.5 mV ($N = 25$). In fluorescence measurements the effects of changes in extracellular pH or CO_2 and HCO_3^- on intracellular pH were examined [changes in emission ratio (Δ ratio) of BCECF dye are given as a measure of intracellular pH]. AcVE cells were acidotic when compared to controls (by unpaired initial emission ratios). Cells were alkalinized by an increase in extracellular HCO_3^- and CO_2 at constant external pH, but AcVE cells significantly less than Co ($P < 0.01$) indicating a reduced $Na[HCO_3]_3$ cotransporter activity. DIDS ($5 * 10^{-4}$ M), a stilbene inhibitor of the cotransporter, alkalinized both types of cells equally and prevented further cell alkalinisation by an increase in extracellular HCO_3^- and CO_2 at constant external pH. The present data suggest that the apical Na/H rather than the basolateral $Na[HCO_3]_3$ cotransporter was primarily inhibited during AcVE. Further experiments are needed to confirm this observation.

The action of circulating growth factors tested in primary culture of rat proximal tubular cells. *M.S. Nobes, C.A. Pollock, P.T. Heng, and M.J. Field, Department of Medicine, University of Sydney, Royal North Shore Hospital, St. Leonards, Australia.* Compensatory renal hypertrophy following nephrectomy may be regulated by circulating serum factors and local growth factors. Thus, the effects of addition of specific antibodies against known growth factors to serum harvested from either sham-operated (Sx) or unilaterally nephrectomized (Nx) animals were tested in culture. The cell growth parameters determined after 7 days were cell proliferation or hyperplasia, using cellular thymidine incorporation, and increased cell size or hypertrophy, using cellular protein content. Epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) induced hyperplasia ($P < 0.0001$ and $P < 0.01$ respectively), while transforming growth factor β_1 (TGF-β_1) potently inhibited hyperplasia ($P < 0.0001$). IGF-1 also induced hypertrophy ($P < 0.01$). All cell growth effects were neutralized by each growth factor's specific antibody (Ab). Compared to 10% serum from Sx rats, 10% serum from Nx rats significantly enhanced hyperplasia ($P < 0.01$) and hypertrophy ($P < 0.0001$). The addition of anti-EGF Ab to either sera did not affect hyperplasia or hypertrophy. The addition of anti-IGF-1 Ab to Sx and Nx sera did not affect hyperplasia, but did reduce hypertrophy by 12% ($P < 0.02$) and 30% ($P < 0.001$), respectively. Similar effects were noted with anti-IGF-1 Ab + anti-EGF Ab to those using anti-IGF-1 Ab alone, except that Nx serum-induced hyperplasia was reduced by 36% ($P < 0.02$). Conversely, the addition of anti TGF-β Ab to Sx and Nx sera increased hyperplasia similarly by 63% ($P < 0.001$) and 59% ($P < 0.01$), respectively, yet did not alter hypertrophy. In conclusion, IGF-1, EGF and TGF-β_1 have direct effects on rat renal tubular cell growth that can be neutralized by their specific antibodies. Growth promotion by serum from normal rats is likely to involve IGF-1-induced hypertrophy and be limited by TGF-β-induced antiproliferation. Augmented growth by serum from rats undergoing compensatory renal hypertrophy most likely involves enhanced hypertrophy due to IGF-1, and cell proliferation due to both IGF-1 and EGF despite TGF-β-induced antiproliferation.

Effect of serum on secretion of prostacyclin and endothelin-1 by decidual endothelial cells from human pregnancies. *J. Rowe, S. Campbell, T. Hawkins, and E.D.M. Gallery Department of Renal Medicine, Royal North Shore Hospital, St. Leonards, NSW, Australia.* It is widely felt that maternal endothelial cells are centrally involved in pre-eclampsia, and that a factor(s) in serum from women with pre-eclampsia alters endothelial cell function. The purposes of this study were: (a) to examine secretion of vasoactive substances by decidual endothelial cells from normal and pre-eclamptic pregnancies, in response to serum from gestation-age-matched normal and pre-eclamptic women; (b) to compare them with umbilical vein endothelial cells, widely used as a general surrogate endothelial cell; and (c) to determine whether responses are amplified by 48 hours-pre-incubation for in test sera. Endothelial cells isolated from umbilical veins (HUVEC), and from decidual biopsies collected at caesarean section delivery, from both normal (N DEC) and pre-eclamptic (PE DEC) women, were maintained in culture until passage 2, when their secretion of the vasodilator prostacyclin (measured as its stable metabolite, 6 keto PGF1α), and the vasoconstrictor endothelin-1 was compared, in the presence of serum from pre-eclamptic (PE) or gestational age-matched normal (N) pregnant women. Prostacyclin secretion (pg/10^6 cells/24 hr) was higher by all endothelial cell populations incubated in serum from pre-eclamptic women, than in serum from normal pregnant women (Group A: no pre-incubation; Group B: pre-incubation)

Cells serum	*N*	HUVEC PE	*N*
(A) 24 hr	840 ± 101	1172 ± 160	250 ± 70
(B) 24 hr	826 ± 185	1186 ± 254	235 ± 111
(Mean % change A-B)	−2%	+1%	−6%

Cells serum	N DEC PE	*N*	PE DEC PE
(A) 24 hr	360 ± 55	289 ± 186	541 ± 83
(B) 24 hr	306 ± 87	377 ± 100	790 ± 180
(Mean % change A-B)	−15%	+30%	+46%

Pre-incubation of PE DEC (% change A-B) in pre-eclamptic serum amplified prostacyclin secretion ($N < 0.01$). Values for endothelin were not different in serum from normal or pre-eclamptic women. Pre-eclamptic serum contains a factor(s) which stimulates PGI_2 secretion from HUVEC and DEC. Cells from pre-eclamptic women were more susceptible to perturbation of secretion by this factor. Pre-eclamptic serum did not specifically affect endothelin-1 secretion.

Monocyte chemoattractant protein-1 mRNA expression in rat kidney proximal tubules cultured in protein-concentrated media. *J. Chen, L. Chen, Y.-Y. Ng, Y.-C. Tay, D.C.H. Harris, Department of Renal Medicine, Westmead Hospital, NSW, Australia.* Cytokines play a pivotal role in synthesis and deposition of kidney extracellular matrix in chronic renal disease. The proinflammatory properties of monocyte chemoattractant protein (MCP-1) make it an ideal candidate cytokine for the production of interstitial inflammation. To investigate the possible role of proteinuria in inducing proximal tubular (PT) cytokine production, cytokine mRNA levels were measured by Northern blot in rat PT isolated by collagenase digestion and Percoll gradient centrifugation. PT were cultured (PTCC) for 5 days to confluence in plastic dishes coated with rat tail collagen in Dulbecco's Modified Eagle's medium and nutrient F-12 Ham (1:1), and then in serum-free medium containing a variety of proteins. MCP-I mRNA was produced by PTCC following 4 or 8 hrs exposure to bovine serum albumin (BSA, 3.7–30 mg/ml), delipidated BSA (dBSA), apotransferrin and holotransferrin (Tf, Tf-Fe, 2–8 mg/ml), heat-inactivated rat plasma (50–100%) but not nitrilotriacetate-Fe (200 μM). MCP-I mRNA expression peaked within 2–4 hrs of dBSA exposure, was maintained for at least 48 hrs with continued exposure, and was reduced within 4 hrs of dBSA removal. Fibronectin and transforming growth factor β mRNA expression was detected in PTCC, but not increased by exposure to protein. In summary, MCP-I mRNA expression is induced by proteins in concentration found in proteinuric urine, and is independent of iron and cytotoxicity. This effect could explain the link between proteinuria and interstitial inflammation in chronic renal disease.

Prostanoid secretion by normal and hypertensive placental villous trophoblasts. *E.D.M. Gallery, Z.Q. Ding, J. Rowe, M.J. Sinosich, D.M. Saunders, Department of Renal Medicine, O&G, Royal North Shore Hospital, NSW, Australia.* Placental villous trophoblasts are thought to be implicated in the pathogenesis of pre-eclampsia. We have developed methods for isolation and culture of these cells. The aim of this study was to compare the secretion of prostanoids by villous trophoblasts from normal pregnancies and those complicated by pre-eclampsia or chronic essential hypertension. Placentas were collected at delivery from normal (N, $N = 8$), pre-eclamptic (PE, $N = 8$) and chronically hypertensive (CH, $N = 5$) pregnant women, and villous trophoblasts isolated and purified as described previously. Cells (1×10^6/ml) were cultured (serum-free

DMEM, 37°C, humidified 5% CO_2/95% air) on Matrigel-covered coverslips. Media was changed every second day and supernates assayed for prostacyclin (PGI_2), prostaglandin E2 (PGE_2) and thromboxane (TXB_2) by radioimmunoassay. Trophoblasts secreted low amounts of PGI_2, PGE_2 and relatively larger amounts of TXB_2. Cells from all groups secreted diminishing amounts of prostanoids as they matured from cyto- to syncytio-trophoblasts over 7 days in culture. TXB_2 secretion by PE trophoblasts was significantly greater than by N trophoblasts ($P < 0.001$). There was no difference in TXB_2 secretion between trophoblasts from N and CH pregnancies, or in PGI_2 or PGE_2 from all three patient populations.

	Day 1		
	N	PE	CH
TXA_2	309 ± 108	1236 ± 428[a]	122 ± 67
PGI_2	8 ± 10	9 ± 12	5 ± 3
PGE_2	16 ± 15	14 ± 13	5 ± 7

	Day 3		
	N	PE	CH
TXA_2	18 ± 6	183 ± 193[a]	9 ± 8
PGI_2	0.6 ± 0.8	1.7 ± 2.7	0.7 ± 0.3
PGE_2	1.5 ± 1.9	1.4 ± 1.9	0.6 ± 0.8

In conclusion, since substances secreted by cytotrophoblasts have access to the maternal circulation, the elevated secretion of thromboxane by PE cells could be involved in initiating the maternal coagulation and endothelial cell damage characteristically seen in pre-eclampsia.

Regulation of glycine tRNA synthetase by TGF-β in cultured human mesangial cells. *J. Williams, S. Osvath, T. Khong, M. Pearse, and D. Power, Department of Clinical Immunology, St Vincents Hospital, Fitzroy, Victoria, Australia.* In studies whose aim was to identify mRNA species regulated by cytokines in human mesangial cells, a previously unknown cDNA fragment was isolated. By Northern blot the cDNA hybridized with a transcript of 2.6 kb. Sequencing of clones obtained from cDNA libraries revealed a predicted open reading frame of 685 amino acids which was 60% identical to silk moth glycine tRNA synthetase (GRS), the enzyme which charges tRNA with glycine, the dominant amino acid in collagens. Expression of this species in bacteria produced a protein of 90 kDa, including the 13 kDa protein tag introduced by the expression vector, which was immunoprecipitated by a polyclonal rabbit antiserum against 20 amino acids from the predicted sequence and by a human autoantibody against GRS (Dr. I. Targof). Bacterial lysates from the cells expressing putative GRS also had 10-fold greater glycine tRNA synthetase activity than control cell lysates. This species is, therefore, believed to encode human GRS. By Northern blot analysis, GRS mRNA transcript numbers in mesangial cells were increased three-fold by stimulation with the fibrogenic cytokine transforming growth factor-β. Regulated expression of GRS is likely to be a response to an elevated requirement of tRNA molecules charged with glycine for the synthesis of collagen. GRS is, therefore, another molecule whose expression may be increased as part of the fibrotic response. To characterize the mechanism of regulation, a genomic clone has been isolated which contains the 5′ end of the cDNA species; studies required to isolate the promotor are continuing.

Differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) in the study of glomerular disease. *K. Paizis, S. Osvath, T. Khong, J. Williams, M. Pearse and D. Power, Immunology Research Centre, St. Vincent's Hospital, Fitzroy, Victoria, Australia.* Whatever the initiating event in glomerulonephritis, many studies have shown that cytokines and other inflammatory molecules contribute to the subsequent pathogenesis of the renal lesion. It is not clear, however, whether there are any mediators produced only by glomerular cells. To determine whether this is the case, we have used the new technique of DDRT-PCR to compare mRNA obtained from the isolated glomeruli of rats with passive Heymann nephritis (PHN) and normals, searching for novel mRNA species. The PHN model was chosen because there is no significant leukocyte infiltrate to complicate analysis. PHN was induced in Sprague-Dawley rats with two injections of anti-Fx1A. At 5 days, rats with PHN had 30–170 mg/24 hours of proteinuria whereas normals had <1 mg/24 hours. Glomerular preparations were prepared by differential sieving. Contamination with tubular fragments was approximately 10%. mRNA was prepared from total RNA using oligo-dT magnetic beads and pools were then made from 3 rats to minimize variation between animals. Two separate pools of mRNA from PHN rats and two from normals were analyzed to assess reproducibility of the results obtained by DDRT-PCR. DDRT-PCR was performed using a variety of 3′-primers and 5′-primers and the bands displayed on sequencing gels. Reproducibility of displays was excellent. Approximately 6,000 separate cDNA species have been compared so far and there were no consistent differences between PHN and control groups. Since each cell type is considered to possess about 10–20,000 different mRNA species, about 40% of the mRNA species present may have been screened. The absence of any differences suggests that a change in the concentration of an individual mRNA species in PHN kidneys may not be detectable unless the diseased cells can be separated from the normal cells. This finding has important implications for the increasing use of this assay in comparing normal and diseased tissues.

Type VIII collagen is produced by and is a structural constituent of normal mammalian glomeruli. *N.S. Greenhill, B. Rüger, Q. Hasan, P.F. Davis, R.P. Murray-McIntosh, P.R. Dunbar and T.J. Neale, Department of Medicine, Wellington School of Medicine, Wellington South, New Zealand.* We have reported that connective tissue mast cells and foreskin and gingival fibroblasts produce type VIII collagen. This short chain collagen variant is a prominent interstitial component in human diabetic nephropathy. Using monoclonal antibodies (6A2, 9H3), specific for the α1 chain of type VIII collagen, enhanced immunohistochemistry has shown that this collagen is localized to paramesangial and subendothelial sites in normal adult bovine, fetal and adult rat, fetal and adult ovine and adult human glomeruli. Two polyclonal antibodies raised against the α1 and the α2 type VIII collagen chains prepared from ovine Descemet's membrane showed strikingly different localizations, e.g. in ovine kidney, optic nerve and aorta, suggesting that the α1 and α2 chains exist as two distinct homotrimers rather than as a single heterotrimer. Pepsin extracts of isolated normal human glomeruli immunoblotted with all four antibodies, yielded reactive polypeptides of 101, 76 and 55 kDa. Cultured rat and human mesangial cells stained positively for α1 and α2 chains and secreted a 61 kDa α2 polypeptide into the culture media. However, under the culture conditions used, the α1 variant was retained intracellulary in both cell lines as degraded limit polypeptides of 40 and 31 kDa. Using synthetic oligonucleotide *in situ* hybridization, mRNA of α1 type VIII collagen was localized in normal human kidney to interstitial cells (mast cells and fibroblasts) but not to glomeruli. RT-PCR did not produce α1 type VIII collagen cDNA products from normal human kidney, but consistently did so from diabetic kidney in which the protein also showed increased expression. We conclude that in the normal glomerulus type VIII collagen is a structural component of mesangial and subendothelial areas contributing to the integrity of the glomerulus and is presumably turned over very slowly. In response to injury (e.g. diabetes, IgA nephropathy) increased message and protein result. Our data suggest that the α1 and α2 chains are individual proteins possibly with separate functions and that the mesangial cell produces both chains.

Abnormalities in type IV collagen in the glomerular basement membrane of bull terrier hereditary nephritis. *H. Brooks, G. Jennings, A. Hendtlass, J. Hood, C. Huxtable, W. Robinson, and J. Savige, Molecular Medicine, University, Department of Medicine, Austin Hospital, Melbourne, Victoria, and Veterinary School, Murdoch University, Perth, WA, Australia.* Bull terrier hereditary nephritis is an autosomal dominantly-inherited disease with a lamellated GCBM identical to that seen in human X-linked Alport syndrome. However unlike Alport syndrome, both the Goodpasture and Alport antigens are present in the GCBM of animals with Bull terrier hereditary nephritis. The aim of this study was to determine the abnormal basement membrane protein in this disease. The distribution of the major GCBM proteins and alpha 1(IV) and alpha 2(IV) collagen chains were determined using immunohistochemistry on tissues sections from affected and normal dogs; and amounts were quantitated using extracted GCBM proteins in an ELISA. The collagen chains were further examined using SDS-polyacrylamide gel electrophoresis. There were no significant differences in the distribution or amounts of laminin, fibronectin, heparin sulphate or nidogen, in the GCBM of affected and unaffected

dogs with immunohistochemistry or between the amounts of these proteins or type IV collagen chains in the ELISAs. However, the 28 kDa monomer corresponding to the non-collagenous domains of the alpha 3 or 4 chains of type IV collagen appeared to be absent from the collagenase-digested affected GCBM in a 15% polyacrylamide gel. The mutation in bull terrier hereditary nephritis probably affects the gene for the alpha 3(IV) or alpha 4(IV) collagen chain; and this disease represents a model for autosomally inherited Alport syndrome and allows us to investigate how an abnormal collagen chain interferes with the structure of the GCBM.

Basic fibroblast growth factor expression in a remnant kidney model of glomerulosclerosis. *A. Tzanidis, A.M. Walker, A.N. Stein-Oakley, N.M. Thomson, Department of Medicine, Monash Medical School, Alfred Hospital, Melbourne, Australia.* Glomerular hypertrophy and subsequent accumulation of extracellular matrix proteins are characteristic features of progressive glomerulosclerosis. The present study sought to describe the role of basic fibroblast growth factor (bFGF) in the development of disease, in an experimental remnant kidney model of glomerular sclerosis and interstitial scarring. Male inbred SD rats underwent either 7/8 nephrectomy by infarction (Nx) or sham laparotomy (Sh). Animals were killed at regular intervals over a 12 week period and 4 to 5 animals were assessed at each time point. The tissue expression of bFGF protein was determined by immunocytochemistry on snap-frozen tissue sections. bFGF messenger RNA was measured by a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique. Reduction nephrectomy (Nx) resulted in early glomerular hypertrophy (week 1 post-Nx) and the development of focal and segmental glomerulosclerosis and interstitial damage beyond the 3rd postoperative week. In normal tissue immunohistochemical studies demonstrated a cellular localization of bFGF protein within glomeruli. Vascular expression was also noted with some outer medullary vessels showing nuclear bFGF staining. There was also strong interstitial cellular bFGF expression between tubules within the inner medulla. Sham operated animals demonstrated normal bFGF expression. In diseased animals, glomeruli demonstrated (as early as 1 week post-nephrectomy) a diffuse staining for bFGF of mesangial matrix which coincided with the onset of glomerular hypertrophy but not with the development of glomerular sclerosis. bFGF was also expressed by glomerular parietal epithelial cells. In addition to this increase in glomerular bFGF, there was a generalized increase in interstitial cellular staining for bFGF throughout the cortex and medulla with marked increases in areas of interstitial fibrosis and tubular damage. No changes in vascular staining were noted. Initial RT-PCR studies of renal tissue throughout the development of disease showed a 1.5–3-fold increase of bFGF mRNA as from the 3rd postoperative week compared to normal controls. These results suggest bFGF may be implicated in the initial hypertrophic response of the remnant kidney and in the pathogenesis of interstial damage of progressive renal disease.

Cytotoxicity of reabsorbed protein in proteinuric chronic renal disease. *L. Chen, J. Chen, Y.-C. Tay, Y-Y. Ng, and D.C.H. Harris, Department of Renal Medicine, Westmead Hospital, NSW, Australia.* Proteinuria is a marker of poor prognosis in progressive renal disease, and proteinuria *per se* may cause renal injury. The toxicity and mechanisms whereby individual proteins cause proximal tubule injury have not been defined. In the present study, primary culture of proximal tubular (PT) cells was employed to differentiate the effects of individual proteins. Proximal tubule segments were isolated by collagenase digestion of rat renal cortex followed by Percoll density gradient centrifugation, and were plated onto plastic culture dishes coated with rat-tail collagen in a mixture of Dulbecco's Modified Eagle's medium and nutrient F-12 Ham (1:1). Individual proteins were added to confluent PT cells in serum-free medium on day 5. Cell toxicity was assessed by LDH leakage into the medium and electronmicroscopy, and lipid peroxidation by malondialdehyde (MDA) production. Both LDH leakage from and MDA production in cells treated with transferrin-Fe (200 μM) for 8 hours and 12 hours in pH 6.0 (to allow dissociation of Fe from transferrin) were significantly ($P < 0.001$; $P < 0.01$ respectively) higher than control (pH 6.0). No toxicity or peroxidation was seen in cells treated with transferrin-Fe (200 μM) in pH 7.4, transferrin (200 μM) or albumin (30 mg/ml) in both pH 6.0 or 7.4. Fe-induced peroxidation occurred before LDH leakage, and Fe-induced toxicity was reduced by procysteine, a natural scavenger of reactive oxygen species. Results with transferrin-Fe (pH6) were similar to those obtained previously with nitrilotriacetate-Fe. These results suggest that: (1) Fe, which is released from transferrin at low pH (similar to that of PT lumen), is responsible for the toxicity induced by transferrin-Fe in PT cells; (2) apotransferrin and albumin are not toxic to PT cells; and (3) Fe causes PT cell damage by lipid peroxidation.

Interstitial myofibroblasts (MF) in experimental renal infection and scarring. *T.D. Hewitson, H. Wu, G.J. Becker, Department of Nephrology, Royal Melbourne Hospital, Victoria, Australia.* Although fibroblasts from diseased kidneys have been studied extensively *in vitro,* almost no data exist on fibroblasts *in situ.* We have examined the temporal and spatial distribution of myofibroblast like cells, a phenotype with fibroblast and smooth muscle features, in an experimental model of renal infection. *E. coli* ($\times 10^5$) were inoculated directly into the renal cortex of Sprague Dawley rats (270 g). Saline was substituted in a control group (C). Animals were sacrificed at 5 time points up to day (D) 24 (*E. coli* $N = 8$, C $N = 3$ each interval). MF were identified by morphology and immunohistochemistry for ∝smooth muscle actin (∝SMA) and compared with staining for MØ (ED-1) and collagen III. Nuclear bromodeoxyuridine (BrdU) incorporation was used with ∝SMA to identify DNA replication in MF. Histological changes included a focal lesion in *E. coli* animals. Interstitial ∝SMA staining was confined to spindle shaped cells resembling MF. Percent fractional area (FA) of ∝SMA staining in the lesion increased from 0.12 ± 0.09 D1 to 20.0 ± 7.1 at D3 ($P < 0.005$), decreasing progressively to 2.0 ± 2.6 by D24. This paralleled BrdU incorporation in MF at D1 0.4 ± 0.5, D3 97.3 ± 37.5, and D24 2.6 ± 2.2 cells/0.25 mm^2. ED-1 +ve cells increased from 371 ± 185 D1, to 894 ± 88 at D3 ($P < 0.005$), declining to 230 ± 108/0.25 mm^2 by D24. Intracellular collagen III and ∝SMA staining was co-localized in spindle shaped cells at D3. FA of collagen III increased by D24 ($P < 0.05$). In conclusion, MF accumulate transiently during renal interstitial fibrosis and are derived at least in part from local proliferation. Time course parallels that of MØ infiltration. Double labelling suggests that MF may be synthetically active.

Both glomerular and interstitial injury are induced by anti-Tamm-Horsfall protein (THP) antibodies. *Q. Song, D.J. Nikolic-Paterson, R.C. Atkins, and H.Y. Lan, Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* Several groups have previously described tubulointerstitial nephritis (TIN) induced by the injection of anti-THP antibodies into experimental animals. However, little data are available regarding glomerular changes in this model. The aim of this study was to examine the nature of glomerular changes in a model of TIN. Rats were injected i.v. with 5 mg purified sheep anti-rat THP IgG once a week for up to 5 weeks. Groups of 5 rats were killed on day 1 and on weeks 2, 4, 6, 8 and 10. Urinary protein excretion increased from 10.1 ± 3.2 mg/day on week 2 (normal 3.9 ± 2.2 mg/day) to 98 ± 35.4 mg/day by week 10 ($P < 0.05$ vs. normal). Animals also developed a T cell response to THP as demonstrated by a positive skin DTH reaction which was absent in normal rats. In the interstitium, granular deposition of sheep IgG, rat IgG and C3 was apparent on the tubular basement membrane of THP positive tubules. This deposition was maximal by week 4 and remained strong until week 10. Associated with sheep IgG deposition was a focal intense leukocytic infiltrate. However, renal injury was not restricted to the interstitium as previously suggested. In the glomerulus there was also granular deposition of sheep IgG, rat IgG and C3 on the GBM. This deposition was maximal at week 4 and was associated with a mild glomerular macrophage infiltrate and declined thereafter. In conclusion, these results demonstrate that injection of anti-THP antibodies induces both immune complex glomerulonephritis and TIN. Hence, caution must be used when interpreting results from anti-THP antibody studies as pertaining to only interstitial disease.

Glomerular tuft hypertrophy precedes glomerulosclerosis in rats treated with puromycin aminonucleoside. *M.M. Cahill, J.F. Bertram, and G.B. Ryan, Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, Australia.* Recent interest has focused on the role of glomerular hypertrophy in the development of focal and segmental glomerulosclerosis (FSG). We used unbiased stereological methods to investigate whether glomerular hypertrophy precedes, accompanies, or follows the development of FSG in a puromycin aminonucleoside (PAN) model previously believed to involve no glomerular hypertrophy. Female Sprague Dawley rats weighing 200 g received subcutaneous injections of PAN (2 mg/100 g bodyweight) at weeks 0, 1, 2, 4, 6, 8 and 10. Control rats

received an equivalent volume of normal saline. Kidneys were perfusion fixed at weeks 7 or 13. Tissue was embedded in glycolmethacrylate and glomerular tuft volume estimated as described by Bertram et al. Glomerular tuft volume (mean ± SD) in PAN-treated rats at week 7 was 48% greater than in saline-treated rats [10.45 ± 2.58 ($N = 6$) versus 7.05 ± 1.07 ($N = 8$) $mm^3 \times 10^{-4}$]. At week 13, glomerular volume was 63% greater in PAN-treated rats than in saline-treated rats [12.85 ± 2.90 ($N = 7$) versus 7.90 ± 0.80 ($N = 8$) $mm^3 \times 10^{-4}$]. Two-way analysis of variance indicated significant effects of PAN ($P < 0.001$) and time ($P < 0.05$) on glomerular volume. Glomerulosclerosis, assessed in paraffin sections stained with PAS, was absent at 7 weeks and minimal at 13 weeks. These results indicate marked glomerular tuft hypertrophy in a PAN model of FSG. In addition, the results indicate that glomerular hypertrophy precedes FSG in this model. In studies in progress we are assessing the contribution of glomerular matrix expansion and glomerular capillary growth to this glomerular hypertrophy.

Podocyte morphology in puromycin aminonucleoside-treated rats co-administered tungsten or allopurinol. *S.D. Ricardo, J.F. Bertram, and G.B. Ryan, Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, Australia; Division of Nephrology and Hypertension, Milton S. Hershey Medical Center, Hershey, Pennsylvania, USA.* Numerous studies have implicated reactive oxygen species (ROS) in the structural and functional alterations seen in the glomeruli of rats treated with puromycin aminonucleoside (PAN). Several studies have suggested that the xanthine oxidase pathway is the source of the ROS, but the evidence has primarily been based on the observation that administration of allopurinol (a xanthine oxidase inhibitor) to PAN-treated rats reduces glomerular injury. However, allopurinol and its chief metabolite oxypurinol have antioxidant properties. In the present study, we compared the effects of allopurinol and tungsten (a specific xanthine oxidase inhibitor) on podocyte ultrastructure, renal xanthine oxidase and xanthine dehydrogenase activity, and urinary protein excretion in PAN-treated rats. Female Sprague-Dawley rats received a single i.v. injection of either PAN (5 mg/100 g body wt) or an equivalent volume of saline. Co-administration of allopurinol (single i.p. injection 4 hours before PAN) to PAN-treated rats reduced proteinuria by approximately 70% over the 10 days (day 0 to day 10) studied, and reduced podocyte foot process effacement as assessed by electron microscopic morphometry by approximately 40%, compared to rats that received PAN alone. Co-administration of allopurinol to PAN-treated rats did not reduce xanthine oxidase activity, but did reduce the combined activity of xanthine oxidase and xanthine dehydrogenase on day 5. Co-administration of tungsten (0.7 g/liter of drinking water from 7 days before PAN injection to sacrifice) to PAN-treated rats did not reduce either proteinuria or foot process effacement. Rats co-administered tungsten and PAN had significantly reduced renal xanthine oxidase and combined xanthine oxidase and xanthine dehydrogenase activities on days 5 and 10, compared to rats treated with PAN alone. These results provide evidence that the structural and functional protection provided by allopurinol in PAN-treated rats is due to the antioxidant properties of allopurinol, rather than to its activities as a xanthine oxidase inhibitor. Alternative sources of ROS in PAN-treated rats must therefore be considered.

Increased expression of TGFβ1 mRNA in a remnant kidney model of glomerulosclerosis. *A. Tzanidis, A.M. Walker, and N.M. Thomson, Department of Medicine, Monash Medical School, Alfred Hospital, Melbourne, Australia.* Chronic renal injury is characterized by progressive accumulation of extracellular matrix (ECM) proteins within glomeruli. Although many cytokines may be involved in this process, TGFβ1 is likely to be a major player in the regulation of ECM production and the development of glomerulosclerosis. This study describes the role of TGFβ1 in the rat remnant kidney model of glomerulosclerosis and interstitial scarring and the influence of Angiotensin I converting enzyme (ACE) inhibition on the expression of TGFβ1. Male inbred SD rats were randomly allocated into three groups: Group 1 animals underwent 7/8 nephrectomy by infarction (StNx); Group 2 animals underwent 7/8 nephrectomy and were treated with enalapril (ENx); and Group 3 underwent a sham nephrectomy (StSh). Renal function, urinary protein excretion and systolic blood pressure were monitored for a 12 week period. Animals were killed at regular intervals over a 12 week period and TGFβ1 messenger RNA (mRNA) was quantified by Northern blot analysis. Reduction nephrectomy (StNx) resulted in early glomerular hypertrophy (week 1 post-Nx) and the development of focal and segmental glomerulosclerosis from the third postoperative week. ENx demonstrated a reduction in glomerular sclerosis score by week 12 compared with the StSh group ($P < 0.01$). Sham operated animals did not develop glomerulosclerosis. TGFβ1 mRNA levels increased with disease progression, with a 4-fold increase by week 12 ($P < 0.005$) compared to sham controls. TGFβ1 mRNA levels in enalapril treated animals also rose by week 12 ($P < 0.005$), but this rise was significantly less than that seen in the StNx group ($P < 0.005$). TGFβ1 mRNA levels did not change in StSh control animals. These data suggest a possible role for TGFβ1 in the development of the sclerotic changes seen in this experimental model of glomerulosclerosis, and that the protective effect of ACE inhibition may be secondary to the reduced expression of TGFβ1.

EGF and tubular proliferation in experimental glomerulonephritis. *G.H. Tesch, H.Y. Lan, R.C. Atkins, and D.J. Nikolic-Paterson, Department of Nephrology, Clayton, Victoria, Australia.* The relationship between renal epidermal growth factor (EGF) production and tubular proliferation is unclear. Studies of experimental acute tubular necrosis and ischemic injury have found that EGF synthesis is down-regulated during the period of epithelial cell proliferation which follows tubular injury, yet EGF is known to be a growth factor for tubular epithelial cells both *in vitro* and *in vivo*. EGF expression and tubular proliferation were examined in anti-Thy-1 nephritis by means of two-color immunohistochemistry staining of cryostat tissue sections. Groups of 5 rats were injected with a single intravenous dose of 5 mg/ml OX-7 IgG and then killed on days 1, 4, 6, 8, 10, 14, 21 and 28. In addition to the well-described mesangial lesion, mild tubular injury was evident in this model. While distribution of EGF expression was unaltered during the disease, the level of tubular EGF mRNA and protein expression was reduced by 30–50% over days 1–14, returned to normal levels on day 21, and rebounded to levels 2-fold greater than normal on day 28. There was a significant increase in the percentage of proliferating (PCNA+) cortical tubular cells on day 8 of the disease ($P < 0.05$)—the time of minimal EGF expression—which returned to normal levels by day 14. The increase in epithelial cell proliferation was restricted to tubules lacking EGF expression (mostly proximal tubules), with no change in proliferation of the EGF+ tubules. However, on day 28 there was an increase in proliferation in EGF+ tubules (but not EGF− tubules) coincident with the time of the rebound in EGF expression. EGF-receptor (EGF-R) expression was also analyzed by immunohistochemistry. In normal kidney there was strong EGF-R expression within the glomerulus (mostly podocytes) and weak expression by EGF+ tubules. There was no change in tubular EGF-R expression during the disease. In conclusion, these results suggest that proliferation of EGF+ tubules is related to the level of EGF expression and that proliferation of EGF− tubules appears to be unrelated to renal EGF expression.

The tissue inhibitor of the metalloproteinases type I (TIMP-1) in experimental nephrosis. *C.L. Jones, J. Forbes, A. Haralambous-Gasser, K. Kelynack, R. Walker, T. Hewitson, and G. Becker, Victorian Paediatric Renal Service, Royal Children's Hospital and Royal Melbourne Hospital, Parkville, Victoria.* Previous studies of rats developing renal interstitial fibrosis with nephrosis, induced by the aminonucleoside of puromycin (PAN), have found increased amounts of the tissue inhibitor of the metalloproteinases type I (TIMP-1) in the kidneys. The aim of these studies was to determine the location of TIMP-1 and the matrix metalloproteinase (MMP) enzymes that TIMP-1 inhibits. An acute model of PAN was created in female Sprague Dawley rats by performing a unilateral nephrectomy and 1 week later giving an intraperitoneal (i.p.) injection of 15 mg/100 g body weight of PAN in experimental rats and i.p. vehicle alone in control rats. Groups ($N = 5$) of PAN and control rats were sacrificed at 1, 2, 4 and 5 weeks. Northern blotting and *in situ* hybridization for the mRNA for TIMP-1, and immunohistological and zymography demonstration of MMP1, MMP2 and MMP3 were performed. The expression of mRNA for TIMP-1 was increased by 10-fold at 1 and 2 weeks and was reduced towards control levels at 4 and 5 weeks. *In situ* hybridization revealed mRNA for TIMP-1 in the perivascular tissues of major vessels in controls, and extending up small arterioles, including the afferent arteriole, in PAN rats. MMP's were colocalized with TIMP-1 by immunohistology. Functional activity of the type IV collagenases was increased (4-fold control levels at weeks 1 and 2). These results localized TIMP-1 in the kidney for the first time, indicated the potential importance of the perivascular tissues in renal interstitial

fibrogenesis, and demonstrated the inhibitor was colocalized with its potential substrates.

Interstitial mucinosis: Extension of the concept. *J.P. Dowling and J.W. Agar, Anatomical Pathology, Alfred Hospital, Melbourne; and Renal Unit, Geelong Hospital, Geelong, Victoria, Australia.* Renal interstitial mucinosis (IM) was first noted in a patient with acute renal failure (ARF) developing after ingestion of a single dose of Diclofenac on two separate occasions; medullary IM was the main finding on biopsy taken after this second episode. This change, although distinct from the recognized forms of NSAID toxicity, seems to be especially related to the use of NSAIDs but is not specific to these drugs; IM has been seen in two patients with ARF due to mushroom poisoning and in a patient presenting with ARF after paraquat ingestion with subsequent recovery of renal function. The NSAID-related IM may occur in several settings: (1) The ARF presentation within 1–2 days following ingestion of usual doses of medication in which the main pathology is medullary IM. (2) Persistent but varying degrees of renal failure associated with protracted though perhaps intermittent ingestion of NSAIDs; moderate medullary and lesser cortical IM is accompanied by considerable interstitial sclerosis with minor chronic inflammation, and in one instance, papillary necrosis. (3) Hypersensitivity-type acute interstitial nephritis with blood and tissue eosinophilia with IM as a marker for NSAID ingestion. (4) IM associated with invasive transitional cell carcinoma of the renal pelvis subsequently found on questioning to have occurred in a chronic user of Naprosyn. The mechanism of accumulation of mucin (glycosaminoglycan, mostly hyaluronan) is unknown. IM may develop following a cessation of normal physiological mucin excretion in the urine or possibly due to excessive production by renal interstitial cells.

Parenchymal renal veins—A renal Trojan horse. *J.P. Dowling, Anatomical Pathology, Alfred Hospital, Melbourne, Victoria, Australia.* Little attention has been paid to the development of pathology related to the intrarenal arcuate and interlobar veins in descriptions of changes seen in renal biopsies. Observation suggests that renal vein alterations are not uncommon in a variety of glomerulonephritides, related or not to the coagulation abnormalities of the nephrotic syndrome. In occasional instances chronic venous obliteration may be central to progressive deterioration of renal function as illustrated by the following patient: A 51 year-old developed nephrotic syndrome with swollen ankles and a urinary protein of 5.5 g/day with normal renal function; investigations for secondary causes such as lupus erythematosus were negative. Renal biopsy showed stage 2 membranous nephropathy with subepithelial spikes and granular deposits of IgG and C3 predominantly on direct immunofluorescence. One interlobular vein showed near-obliteration related to active cellular thrombophlebitis. No treatment was administered. Approximately two years later persistent proteinuria of 3.5 g/day and a deterioration of renal function to 2.4 mmol/liter were noted and a second biopsy showed a slight advance in the stage of membranous nephropathy without increased glomerular obsolescence; an easily discernible increase in interstitial fibrosis and mild to moderate venous mural sclerosis without obliteration were noted. The latter may have resulted from the previously seen intrarenal thrombophlebitis. Other venous-related pathologies that have been noted on biopsy are thrombosis perse usually in the context of the nephrotic syndrome, partial venous obliteration as demonstrated by elastin stains, linear venous scars following complete obliteration of the vein, and Tamm-Horsfall thrombi related to tubulovenous communication.

Tubulointerstitial damage is a better predictor of progressive renal failure in the rat remnant kidney model. *L.L. Wu and C.J. Roe, University of Melbourne, Department of Medicine, Heidelberg Hospital, Victoria, Australia.* Research suggests tubulointerstitial pathology rather than glomerular sclerosis better correlates with progressive renal failure. This study correlates the sequential glomerular and tubulointerstitial changes with functional outcomes in a rat variable remnant kidney model. Male Sprague-Dawley rats weighing 200–250 g were randomly divided into 5/6 (FSN, $N = 40$) and 1/6 ablation (OSN, $N = 40$). Ten rats from each group were killed at 4, 8, 12 and 16 weeks post-surgery. Remnant kidney weight (Kwt), systolic blood pressure (BP), 24 hour urine protein (U_{Pr}) and serum creatinine (S_{Cr}) were measured at each time point. Tubulointerstitial damage (TID) (tubular dilation, casts, tubular atrophy, interstitial infiltrate, interstitial fibrosis) was quantitated by point-counting and 50 glomeruli per kidney were evaluated semiquantitatively for glomerular sclerosis (GS). The dependency of S_{Cr} on each histological variable was assessed using multivariate analysis. Six FSN and one OSN died. At each time point Kwt, BP, U_{Pr} and S_{Cr} were significantly higher in FSN. There was significantly more GS and TID and less normal tubules and glomeruli in FSN. S_{Cr} was dependent on percentage of remaining normal tubules and amount of interstitial fibrosis using multivariate stepwise linear regression analysis. Group data (mean ± SEM) are in the table.

Group	S_{Cr} *μmol/liter*	Normal tubules (%)	Fibrosis %	GS/50 glom
FSN ($N = 34$)	110 ± 5	53.0 ± 3.2	4.5 ± 0.8	47.6 ± 4.7
OSN ($N = 39$)	60 ± 1	80.6 ± 0.5	0.1 ± 0.0	4.1 ± 0.9
P	<0.001	<0.001	<0.001	<0.001

We conclude that in the rat remnant kidney model there is renal hypertrophy and progressive renal damage. The decline in renal function is predicted by the reduction in normal tubules and the amount of interstitial fibrosis rather than by glomerular sclerosis.

The application of Laser Scanning Confocal Microscopy to human renal pathology. *K. Kelynack, K. Nicholls, J. Bertram, J. Hill, and G. Becker, Department of Nephrology, Royal Melbourne Hospital, Department of Anatomy, University of Melbourne, Department of Surgery, Royal Melbourne Hospital, Victoria, Australia.* While the use of the Laser Scanning Confocal Microscope (LSCM) in the examination of human kidney sections has not previously been widely reported, its advantages over routine histological procedures suggest potential value in renal pathology. One particular advantage of the LSCM is the ability to screen biopsy specimens using a single Hematoxylin and Eosin (H&E) section. Biopsies of kidneys of living-related renal donors were taken at the time of transplantation. A single biopsy section with a minimum of 10 glomeruli per patient was examined using light and confocal microscopy. The parameters determined were glomerular profile area (GPA), mean glomerular volume (MGV), and interstitial area (IA). The same H&E section was used to measure glomerular basement membrane (GBM) thickness on the LSCM to compare with measurements obtained from electron microscopy (EM) using the orthogonal intercept method (epon resin embedded, one glomerulus/patient). A Spearman Rank test for correlation was used for data analysis. Results indicated a poor correlation between point counting on light microscopy (LM) and confocal microscopy for interstitial area ($R^2 = 0.333$). A strong correlation was found for MGV as determined by point counting ($R^2 = 0.952$, $P < 0.01$). A weak correlation for MGV was also found between the image analysis (LSCM inbuilt software) and LM methods ($R^2 = 0.539$). There was no correlation between GBM thickness measurements by the two methods used ($R^2 = 0.115$). We conclude that LSCM does not replace traditional methods of renal morphometric measurement. It cannot be used to determine accurately the GBM thickness in renal biopsy material. However, in the future the LSCM may be used as a diagnostic screening tool for samples requiring EM, provided that absolute numbers are not critical.

Extracellular matrix (ECM) degradation in cyclosporine A nephrotoxicity. *C.L. Jones, J. Fecondo, K. Kelynack, J. Forbes, R. Walker, and G. Becker, Victorian Paediatric Renal Service, Royal Children's Hospital and Royal Melbourne Hospital, Parkville, and Department of Chemistry, Swinburne University, Victoria, Australia.* The mRNA and protein expression of the tissue inhibitor of the metalloproteinases (TIMP-1) was examined in the rat model of cyclosporine A nephrotoxicity (CsAN). A model of CsAN was created using daily intraperitoneal injections of 15 and 25 mg/kg body weight of CsA, and controls received i.p. injections of vehicle alone. Groups of 5 experimental and 5 control rats were sacrificed at 1, 2, 3, 4 and 6 weeks. Northern blotting was performed for TIMP-1, procollagen α1(I), procollagen α1(IV), TGFβ1, and the 72 kDa type IV collagenase. Quantitation of mRNA bands was done by densitometry with respect to the 18S RNA band. The protein expression of TIMP-1 and collagenases were quantitated by immunohistochemistry using point counting. A peptide of TIMP-1 (19 amino acids long) and a peptide common to rat stromelysin and interstitial collagenase (14 amino acids long) were synthesized, coupled to KLH, then the coupled peptide was used to raise

polyclonal antisera in rabbits and affinity purified antibody was isolated using the peptide coupled to Affigel. Increased amounts of mRNA for TIMP-1 were found at all time points (in all cases 2–5 × control amounts). The mRNA for procollagen α1(I) was increased at all time points, but that for procollagen α1(IV) was only increased at weeks 1 and 2 ($P < 0.05$ in all cases). No change in mRNA amounts for TGFβ and type IV collagenase was found. Increased expression of TIMP-1 protein was found on immunohistochemistry, but no increase in collagenase was found (Mann Whitney U test, $P < 0.05$). There was colocalization of TIMP and collagenase in both controls and experimental animals. The location of the TIMP was in the interstitial spaces, little was found in glomeruli, and it was dense about the adventia of vessels. These results support an important role for the inhibition of ECM degradation in the progressive accumulation of scar tissue in CsA.

Treated end-stage renal disease (ESRD) in aborigines in the top end of the Northern Territory (NT), 1978–1993. *W. Hoy, B. Hayhurst, J. Mathews, and D. Pugsley, Menzies School of Health Research, Darwin, NT; and Renal Unit, Queen Elizabeth Hospital, Woodville, SA (DP), Australia.* The NT's Top End ESRD treatment program was started by DP in 1978. Based in Darwin, it serves a 1991 population of 105,405 non-Aborigines (NA) and 25,387 Aborigines (A) over a very large area; most A must move permanently from their families and isolated communities to receive treatment. A total of 83 A and 44 NA had been treated through December 1993, with 46 A and 23 NA alive at that point. New A cases are increasing rapidly, and have exceeded NA cases since 1989. The average annual incidence for 1988–1993 was 38 per million (pm) for NA and 440 pm for A, (358 pm for nonTiwi A, and 1636 pm for Tiwi A). The age-adjusted rate in A was 17.4 times that of NA (14.1 for nonTiwi A, and 59.6 for Tiwi A). Most A with ESRD were much younger than NA (mode 35 vs. 65 years). Male:female ratio was 0.8:1, vs. the male dominance for NA (1.8:1). A had a higher proportion of GN-ESRD (52% vs. 29%), and diabetic ESRD (21% vs. 9%); 5% had amyloid associated with lung infections, TB and leprosy. Most subjects received hemodialysis, and a few received peritoneal dialysis. Twenty-one NA (48%) and 18 A (only 23%) received transplants (TP), with clinical ineligibility being a major obstacle. TP survival was 100% at 5 years for NA, but was 58% and 24% at 2 and 5 years for A; noncompliance, rejection, intensified immunosuppression and exacerbated comorbidities was the common sequence leading to death. In December 1993, 70% of NA but only 13% of A had functioning TPs. Fewer A with ESRD had preexisting vascular disease, but more had chronic lung infections, rheumatic heart disease, TB, hepatitis B, healed leprosy, alcohol abuse, and morbid obesity. Deaths in A were less often due to heart disease than in NA (30% vs. 50%), and more often due to infections (24% vs. 12%), and withdrawal from treatment (23% vs. 6%). Withdrawal reflects poor tolerance or compliance with treatment and lack of social and family supports. High rates of albuminuria and clinical nephropathy in A are compatible with their high ESRD rates. Treatment choices and results reflect their profoundly inferior health status and socioeconomic deprivation. A 2.5-fold increase in A ESRD is projected by the year 2000. Precursors of ESRD must be studied, and widespread screening and renalprotective treatment introduced, along with intensified and innovative efforts to improve the health and welfare of the entire A population.

Acute renal failure after cardiac surgery: Incidence, outcomes and risk factors. *G. Mangos, M.A. Brown, W. Chan, D. Horton, P. Trew, and J.A. Whitworth, Departments of Renal Medicine, Medicine and Cardiothoracic Surgery, St. George Hospital and University of New South Wales, Kogarah, New South Wales, Australia.* Although acute renal failure (ARF) has long been recognized as a potential consequence of cardiac surgery, the incidence of this complication has varied widely in published literature and no Australian data have been gathered to help predict the risks of ARF in patients with pre-existing renal disease. We therefore determined the incidence, outcome and risk factors for ARF in a retrospective case control analysis of 903 consecutive patients who had cardiac surgery (795 CABG, 68 valves, 40 combined valve/CABG) from 1992–1993. ARF was defined as a doubling of serum creatinine to >0.13 mmol/liter if renal function was normal pre-operatively, or else a rise in serum creatinine by ≥0.10 mmol/liter after cardiac surgery. Ninety percent had "normal" pre-operative renal function (serum creatinine ≤0.13 mmol/liter). ARF developed in only 1.1% of these subjects, none required dialysis, and mortality was 0.7%. ARF developed in 16% of those with impaired pre-operative renal function; 20% of these required dialysis and 13% of this whole group died. The risk of ARF rose from 10.4% in those with pre-operative serum creatinine 0.14–0.20 mmol/liter to 36.8% if creatinine was >0.20 mmol/liter ($P < 0.01$). Mortality was significantly higher (4.2% vs. 0.7%, $P < 0.01$) and length of hospital stay longer [15 vs. 9 days (median), $P < 0.001$] in those with impaired pre-operative renal function. Case control analysis showed that ARF was more likely in those over 65 years, if valve surgery was included; cardiopulmonary bypass time was prolonged or LV dysfunction was present pre-operatively. These data confirm that ARF following cardiac surgery is unlikely without impaired pre-operative renal function but has a mortality rate of 13%. Moreover, impaired pre-operative renal function alone is associated with higher mortality and prolonged hospital stay. Pre-operative assessment of renal function should be mandatory in patients having cardiac surgery, and studies to prevent ARF in this setting should focus on the high risk subsets described in this study.

Effect of pre-operative supplementation with α-tocopherol and ascorbic acid on markers of renal and myocardial injury in patients undergoing cardiac surgery. *J. Westhuyzen, S.J. Fleming, D. Cross, M. Frenneaux, F.A. Khafagi, A. Cochrane, P.J. Tesar, and T. Mau, Conjoint Renal Laboratory, Department of Pathology, and Departments of Renal Medicine, Cardiology, and Nuclear Medicine, Royal Brisbane Hospital; Department of Cardiac Surgery, The Prince Charles Hospital, Brisbane, Australia.* Cardiac surgery employing cardiopulmonary bypass (CPB) is associated with oxidative stress. Pretreating patients with α-tocopherol (vitamin E) and ascorbic acid (vitamin C) may augment the patient's antioxidant defenses and confer increased protection against free radical-mediated injury to *inter alia* heart and kidney. We undertook a double-blind, randomized trial of 69 patients undergoing coronary artery bypass grafting, utilizing CPB and monitoring indices of renal and myocardial injury. Subjects were randomly assigned to receive placebo or 750 IU dl α-tocopherol supplements/day for 7–10 days, and 1 g ascorbic acid 12 hours prior to surgery. Plasma α-tocopherol concentrations, raised four-fold by supplementation, fell by 70% in the supplemented group and to negligible levels in the placebo group. Relative to urinary creatinine, the excretion of N-acetyl-β-D-glucosaminidase, adenosine deaminase binding protein, and γ-glutamyl transpeptidase increased significantly following surgery ($P < 0.001$; no differences between groups). There were also no significant differences between the groups with respect to release of creatinine kinase MB isoenzyme, nor in the extent and severity of the myocardial perfusion defect determined by Thallium-201 uptake and ECG analysis. We conclude that pretreating patients undergoing elective cardiac surgery with antioxidant vitamins offers no additional protection to the myocardium or renal proximal tubules than standard practice.

Primary focal sclerosing glomerulonephritis. *L.S. Ibels, D.A. Waugh, R.J. Caterson, J.F. Mahony, and C.A. Pollock, Department of Renal Medicine, Royal North Shore Hospital, New South Wales, Australia.* In an endeavour to determine the natural history and factors of importance in the progression of primary focal sclerosing glomerulonephritis, 54 patients (34 M, 20 F) aged 8–70 (mean 42) years were studied over a period of 1–204 (mean 79) months from presentation. Features at presentation included hypertension (67%), nephrotic range proteinuria (44%), raised serum creatinine (48%), hypercholesterolemia (65%), and a family history of nephritis (15%). Progression to end-stage renal failure occurred in 14 (26%), patients, 1–173 (mean 55) months post-presentation and a >20% decline of renal function occurred in a total of 33 patients (62%). The cumulative renal survival was 79.7% at 5 years and 75.9% at 10 years after presentation. Factors significantly associated with an adverse outcome on initial presentation, included a longer duration of symptoms, hypertension, and age <30 years; on initial laboratory evaluation, the degree of impairment of renal function, the degree of proteinuria, nephrotic range proteinuria, and serum cholesterol concentrations; on renal biopsy, the percentage of glomeruli with global or segmental sclerosis, the degree of tubular atrophy/interstitial fibrosis, the degree of interstitial inflammation and the degree of arteriolar thickening; and during follow-up, the degree of proteinuria and persistence or development of hypertension. Primary focal sclerosing glomerulonephritis may be familial in over 15% of patients and has a guarded prognosis, particularly in patients with heavy proteinuria, hypertension, renal impairment and age <30 years at presentation. Identification of patients with a poorer prognosis should assist in making therapeutic decisions to retard the progression of disease.

Renal tubular acidosis in primary Sjögren's syndrome. *P.T.H. Coates, T.P. Gordon, G. Tallis, and L.J. Barratt. Department of Medicine, Flinders Medical Centre, Clinical Immunology and Clinical Chemistry, Flinders University of South Australia, Bedford Park, Australia.* Interstitial nephritis and a distal renal tubular acidosis (RTA) are reported to occur with primary Sjögren's syndrome (SS). We have used a sensitive measure of distal hydrogen ion secretion to estimate the prevalence of acidification defects SS. Fifteen subjects with primary Sjögren's syndrome were studied with ammonium chloride loading tests and oral sodium bicarbonate loading tests. In the ammonium chloride test, minimal urine pH after a three day oral load was measured (normal results <5.5). After oral sodium bicarbonate load, urine and blood pCO_2, pH and bicarbonate were measured (normal results, pH >7.6, then pCO_2 >70 and urine-blood pCO_2 >20). A group of normal controls were also studied. Nine subjects have evidence of impaired distal hydrogen ion secretion when assessed by bicarbonate loading testing, with urine pCO_2 <70 and urine-blood pCO_2 <20 in an alkaline urine. Two of the nine subjects had complete distal acidosis, one had latent renal tubular acidosis with an inability to secrete an acid load and inability to generate an elevated urine pCO_2. Six subjects were able to secrete acid but unable to generate an elevated urinary pCO_2 (rate related RTA). Six subjects were normal on both tests. Urinary acidification defects are common in primary SS (60%). We have provided the first description of rate related RTA in pSS. These results support the thesis that SS is a generalized disorder of epithelial cells.

Should all children with a urinary tract infection be investigated? *J.C. Craig, L.P. Roy, J.F. Knight, L.M. Irwig, Department of Nephrology, Royal Alexandra Hospital for Children & Department of Public Health, University of Sydney, Sydney, NSW, Australia.* The investigation of all children with a urinary tract infection (UTI) is advocated for the detection of renal tract abnormalities such as vesicoureteric reflux (VUR), urinary obstruction and renal parenchymal abnormality. The data of a prospective cohort of 280 preschool children with their first proven UTI were analyzed to determine whether this expensive and invasive approach is valid. UTI was defined as any growth in a bladder tap or catheter sample and >108/liter in a voided urine sample. A radiological contrast MCU was used for the detection of VUR, which was present in 27% of children (72/265). Renal parenchymal abnormality was defined as an abnormal ^{99m}Tc-dimercapto-succinic acid scan (DMSA). This was present in 40% of children (108/272). 1.1% (3/280) had associated urinary tract obstruction. This diagnosis was considered if there was significant pelvicalyceal or ureteric dilatation on renal tract ultrasonography and confirmed by ^{99m}Tc-diethylenetriamine-pentacetic acid scintigraphy, with pre-hydration and frusemide washout. Multivariate analysis of all variables at presentation by logistic regression confirmed that a history of fever was the strongest predictor of VUR and abnormal renal parenchyma. If children under 3 months were excluded (fever is an unreliable sign of sepsis in young infants), 42 of the 212 older patients (20%) did not have a history of fever. Only 1 child in this group had renal parenchymal abnormality and VUR. The strength of this association between fever and VUR, and fever and abnormal renal parenchyma are given.

	Relative risk (95% CI)	*P* value	Sens. %
VUR	17 (2–130)	<0.0001	98.2
Abnormal DMSA	35 (4–263)	<0.0001	98.8

	Spec. %	Post-test probability if fever present %	Post-test probability if fever absent %
VUR	24	32	2.2
Abnormal DMSA	29.4	46	2.2

These data strongly suggest that children over 3 months of age who present with a UTI without a history of fever are very unlikely to have either VUR or abnormal kidneys. If prospective follow-up of this cohort confirms that the renal tracts of this group remain normal, a renal ultrasound examination to screen for the rare but treatable entity of urinary tract obstruction may be all that is required to investigate this group of children.

A comparison study of amitriptyline vasopressin, and amitriptyline with vasopressin in nocturnal enuresis. *J.R. Burke, Y. Mizusawa, A. Chan, K. Webb, Royal Children's and Mater Children's Hospitals, Brisbane, Australia.* Forty-five children aged 6 to 14 years with primary nocturnal enuresis were randomized to determine whether desmopressin is more effective than amitriptyline, and whether the combination of amitriptyline-desmopressin is more effective than amitriptyline or desmopressin alone. The amitriptyline dosage was 25 mg for children 6 to 10 years and 50 mg aged 10 to 14 years. Desmopressin 20 μg was given for all age groups. After a run in period of two weeks, children were treated for 16 weeks and then observed for 12 weeks. In the amitriptyline group mean wet nights per week decreased from 5.8 ± 0.9 to 3.3 ± 1.9 ($P < 0.0005$); in the desmopressin group mean wet nights per week decreased from 6.0 ± 0.9 to 4.7 ± 1.7 ($P < 0.02$); in the amitriptyline-desmopressin group, mean wet nights per week decreased from 6.3 ± 0.9 to 3.3 ± 2.5 ($P < 0.0006$). When comparing the groups, mitriptyline-desmopressin and amitriptyline were statistically more effective than desmopressin in week 6 ($P < 0.009$), week 8 ($P < 0.03$), and week 10 ($P < 0.04$). No significant side effects occurred. At this dosage, amitriptyline was more effective than desmopressin and the combination of desmopressin with amitriptyline did not confer any additional benefit.

Oxidation of LDL in renal failure. *W.H.F. Sutherland, R.J. Walker, M.J. Ball, S.A. Stapely, M.C. Robertson, Department of Medicine, Otago Medical School, Dunedin, New Zealand.* Renal failure is associated with an increased risk of atherosclerosis. Peroxidation of LDL may be a contributing factor. We determined the susceptibility to copper ion catalysed oxidation, vitamin E content, and chemical composition of LDL isolated from patients with chronic renal failure on haemodialysis (HD 13), or CAPD (7), or with a renal transplant (RT 18) and 15 healthy controls (C). Lag time in conjugated diene formation during oxidation was significantly ($P < 0.011$) shorter (66 min) in LDL from RT mainly due to significantly ($P < 0.01$) shorter lag times in women (47 min, $N = 7$) compared to C (83 min), compared to HD (91 min) and CAPD (82 min). Triglyceride content in LDL was abnormally high (7.3% vs. 5%, $P < 0.001$) in these women compared to controls and was correlated significantly with lag time in RT ($r = 0.5$, $P = 0.034$). Vitamin E content in LDL from RT women was not significantly different from C. The maximum rate of conjugated diene formation (nmol/min/mg) was significantly slower ($P < 0.050$ in all groups (HD 7.44 ± 1.56, CAPD 7.43 ± 2.2, RT 8.28 ± 1.12) compared to C (9.44 ± 1.64) probably due to lower linoleic acid content in LDL particularly in HD and CAPD. Lag time, max rate in diene production and the elevated lipid peroxide content in LDL (45 ± 8 nmol/mg) did not change before, at the end or 24 hours post-HD. These data suggest that dialysis may not alter LDL susceptibility to oxidation, while LDL from RT women is abnormally susceptible to oxidation possibly due to high triglyceride content. This may contribute to the increased risk of atherosclerosis in RT.

Chronic inhibition of neutral endopeptidase by Oral SCH34826 in the rat remnant kidney model. *K. Jandeleit-Dahm, M. Kanazawa, D. Casley, B. Jackson, C.I. Johnston, Department of Medicine, The University of Melbourne, Austin Hospital, Heidelberg, Victoria, Australia.* Atrial natriuretic peptide (ANP) is intimately involved in volume and salt homeostasis. The neutral metalloendopeptidase (NEP, EC 3.4.24.11) degrades ANP. NEP inhibition may promote ANP accumulation, and thus natriuresis. We examined the effect of SCH34826, an orally active NEP inhibitor, in rats with the subtotal nephrectomy model of renal impairment. SCH34826 (90 mg/kg twice daily, P.O) was administered for 3 days to rats ($N = 7$) subjected 4 weeks previously to subtotal nephrectomy, and effects were compared with placebo treatment. NEP in the kidney was inhibited by some 60% (*in vitro* autoradiography method). Urinary ANP (pg/24 hr) increased from 3930 ± 295 to 9094 ± 1089 ($P < 0.01$). In the treatment group there was a natriuresis (pre-treatment 2.03 ± 0.23 mmolNa/24 hrs to 3.18 ± 0.37, $P < 0.01$) not seen in the placebo group, and an increase in protein excretion (279 ± 77 mg/24 hr to 351 ± 123, $P < 0.05$). There were no differences in glomerular filtration rate, systemic blood pressure, or plasma ANP concentration. Chronic SCH 34826 inhibited renal NEP and modulated sodium excretion without apparent systemic hemodynamic effects in this model of renal impairment.

Renal osmolytes in baboons (*Papio hamadryas*). *S.M. Marshall, N.S. Willis, G.G. Duggin, J.S. Horvath, D.J. Tiller, A.G. Gillin, Department of Renal Medicine, Royal Prince Alfred Hospital, NSW, Australia.* Urinary concentration relies on the ability of the inner medullary cells to withstand the high extracellular osmolality during antidiuresis. Various osmolytes accumulate intracellularly to balance extracellular osmolality. Rat and rabbit osmolytes have been widely studied; however, these species may not be good models of human medullary function. Baboons offer a source of primate renal tissue that may better help understand human medullary function. This study aimed to measure the concentration of organic osmolytes in kidneys from baboons and to compare them to similar tissue from rats. Prior to harvesting tissue, animals were allowed water *ad libitum*. Kidneys from 5 baboons *(Papio hamadryas)* and 5 rats (Wistar) were collected following anaesthesia with Ketamine (6 mg/kg i.v.i.) and Pentobarbitone (350 mg i.p.), respectively. Tissue was dissected from the cortex (C), outer medulla (OM) and inner medulla (IM), and weighed. After protein extraction the samples were analysed by an accepted HPLC method for inositol, glycerophosphorylcholine, sorbitol, urea and betaine. The results are reported as mean ± SEM mmol/kg wet weight of tissue. The same organic compounds were detected in baboon renal tissue that were present in rat tissue. The renal distribution pattern of urea and sorbitol were similar in both baboon and rat. Differences in OM concentrations for glycerophosphorylcholine (baboon, 1,556 ± 232; rat, 3,910 ± 150), betaine (baboon, 257 ± 30; rat, 3,680 ± 77) and inositol (baboon, 1,058 ± 82; rat, 2,730 ± 59) were apparent. This HPLC method is suitable for the analysis of organic osmolytes in baboon renal tissue. There are notable distinctions in baboon osmolyte accumulation in the OM. The reasons for these are not clear but may include differences in diet, state of diuresis as well as species differences.

Effect of an antiserum to vasoactive intestinal peptide (VIP) in two rat models of cirrhosis. *M.A. Lonergan, S. Aglibut, and M.J. Field, Department of Medicine, University of Sydney, Concord Hospital, Sydney, NSW, Australia.* It has been proposed that elevated levels of VIP, due to impaired hepatic clearance may contribute to the disturbance of sodium and water homeostasis that occurs in cirrhosis. To explore this possibility, we conducted clearance studies in cirrhotic Sprague-Dawley rats using a purified antiserum to VIP. Two models of cirrhosis were utilized, namely carbon tetrachloride-induced cirrhosis (CCl_4) and common bile duct ligation and resection (CBDL). Animals were studied under Inactin anaesthesia after 16 weeks (CCl_4) and 5–6 weeks (CBDL). The response to an acute saline load was assessed in a protocol involving infusion of Ringer's solution at 4% body weight/hr following a baseline infusion at 1% body weight/hr. VIP antiserum was administered prior to the commencement of the equilibrium period. Sham-treated animals for both models also received the same volume of VIP antiserum. The animals receiving VIP antiserum were compared to control groups receiving acute volume loading alone. Tritiated inulin and ^{14}C p-aminohippuric acid were included in the infused fluids to permit calculation of GFR, fractional electrolyte excretion rates, renal plasma flow and filtration fraction (FF). In the CCl_4 study, infusion of the VIP antiserum resulted in a small increase in the capacity for excretion of the saline load, fractional excretion of sodium (FE_{Na}) rising above baseline by 1.4 ± 0.8% of filtered load ($P < 0.05$), compared to 0.53 ± 0.2 in CCl_4 rats without antagonist. A similar response occurred in absolute sodium excretion. Urine flow also increased over baseline by 13.9 ± 6.4 μl/min/100 g, compared to 3.6 ± 0.14 in the CCl_4 rats without the antagonist. The diuretic response of the antagonist-treated group remained significantly less than that of the sham-treated controls. In the CBDL study, in the presence of VIP antiserum a non-significant fall occurred in sodium and water excretion. In both models, VIP antiserum did not alter the response of the sham-treated group to saline loading. We conclude that VIP may play a role in the sodium and water retention in CCl_4-induced cirrhosis.

Sodium depletion reduces plasma and renal concentrations of the natriuretic hormone vasoactive intestinal peptide. *K.A. Duggan, G.J. Macdonald, and V.Z. Ye, University Department of Medicine, Prince Henry Hospital, Sydney, Australia.* Vasoactive intestinal peptide (VIP) is a 28 amino acid peptide of the secretin glucagon group that causes natriuresis when infused intrarenally or intravenously. Variations in dietary sodium intake have been shown to affect the plasma concentration, metabolic clearance rate and secretion rate of VIP. In this study we sought to determine the effect of sodium depletion on the concentration of VIP in plasma and kidney. Male Sprague-Dawley rats were placed on low (0.008%) or normal (2.2%) sodium diets and drinking water *ad libitum*. A third group were placed on the low salt dict and in addition were given lasix 1 mg/kg/day in the drinking water. After seven days the rats were sacrificed, blood sampled and kidneys harvested. VIP concentrations were determined by radioimmunoassay on unextracted plasma and in kidneys after extraction. There were significant differences between the three groups in the concentration of VIP in both plasma ($P < 0.025$) and kidney ($P < 0.005$). In the group which had received the lasix and low salt diet the renal concentration of VIP (68.34 ± 9.49 fmol/g tissue) was significantly less than both the low sodium (139.82 ± 11.78 fmol/g tissue, $P < 0.001$) and normal sodium (116.95 ± 14.15 fmol/g tissue, $P < 0.01$). In the plasma the concentration of VIP was also lower in the lasix group (12.45 ± 5.74 pmol/liter) than in either the low sodium (53.35 ± 8.60 pmol/liter, $P < 0.01$) or normal sodium (35.02 ± 11.85 pmol/liter, $P < 0.05$). The lower concentrations in the lasix group may be a response to sodium depletion *per se*. Alternately, they may be a response to the presence of a competing agonist as both VIP and lasix increase sodium excretion by affecting the sodium potassium chloride co-transporter in the loop of Henle.

Authentic ouabain is not detected in human plasma. *L.K. Lewis, T.G. Yandle, J.G. Lewis, G.B. Pidgeon, R.J. Kaaja, A.M. Richards, M.G. Nicholls, Endocrinology Department, Christchurch Hospital, Christchurch, New Zealand, and Helsinki University Central Hospital, Finland.* The aim of this study was to develop a method for measuring plasma ouabain levels in healthy volunteers, in patients with disorders of electrolyte balance or arterial pressure, and following ouabain injection, to assist in determining if ouabain is a biologically important circulating hormone. The assay involved extraction of plasma through a C18 column using 25% acetonitrile, 0.1% trifluroacetic acid as the eluting solvent. The eluate was run through an HPLC column (4 to 16% isopropanol gradient), and fractions were analyzed for ouabain content by an indirect ELISA. Cross-reactivity of the locally raised ELISA antibody was ouabain 100%, ouabagenin 77%, strophanthidin 70%, digoxin 1% and less than 0.1% for various adrenal and ovarian steroids and their glucuronides. Assay sensitivity was less than 0.04 nmol/liter. Without HPLC, immunoreactive ouabain was detected in plasma and diluted in parallel with standards, but intra-assay CVs were extremely variable (up to 45%). Furthermore, ELISA after HPLC separation revealed no ouabain in plasma from healthy or pregnant subjects, or in patients with heart failure, renal failure or hypertension of pregnancy, yet ouabain was readily detected in plasma drawn from volunteers at intervals after ouabain injection and in plasma spiked with ouabain down to a concentration of less than 0.1 nmol/liter. In conclusion, plasma from volunteers and various patient groups did not have detectable ouabain as analyzed by ELISA after HPLC, suggesting that the immunoreactive substance in plasma is not authentic ouabain.

Role of the aldose reductase pathway in the pathogenesis of diabetic complication. *H.D. Yan, J.D. Zajac, M.E. Dunlop, and R.G. Larkins, University Department of Medicine, Royal Melbourne Hospital, Victoria, Australia.* The aim of this project is to transfect Chinese hamster ovary (CHO) cells with human aldose reductase (AR) gene as a model system for studying the interaction of the AR pathway and to study the effect of AR overexpression on prostaglandin synthesis. To define more precisely whether overactivity of AR pathway is a major contributor to the pathogenesis of diabetic complications, we have begun characterization of biochemical effects of overexpression of the AR gene in cell culture using molecular genetic methods. The full length hAR cDNA was inserted into the mammalian expression pc DNA-neo vector, which contains CMV promoter and the selection marker, neo. The AR cDNA was also inserted in antisense orientation, or vector alone as controls. CHO cells were stably transfected by high capacitance electroporation with the above constructs. Seventeen sense, 13 antisense and 5 vector alone clones were chosen for measurement of sorbitol using a radioisotopic method for measurement of NADH generated by the action of the enzyme sorbitol dehydrogenase on cellular sorbitol. One of the sense-construct clones has shown a high sorbitol level. To determine whether the overexpression of the AR gene is correlation with high sorbitol levels we have performed Northern blots to measure mRNA expression in the transfected cells. We found that the sense cell line had about 2.5-fold higher basal expression of AR mRNA than found in antisense or vector alone. Furthermore, in a direct AR enzyme assay, measuring NADPH oxidation in the presence of a galactose substrate, this cell line had twofold higher activity (86.59 mU/mg proteins)

in response to the high glucose condition (50 mM) than the controls (43.28 mU/mg proteins). To confirm our hypothesis we measured the prostaglandin (PG) production by using specific radioimmunoassay at low (5.6 mM) and high (25 mM) glucose conditions. We found that the sense cell line had threefold higher PG level (1040.62 pg/mg protein/ml) in high glucose condition than the controls (368.26 pg/mg protein/ml). These results demonstrate that AR overexpression in CHO cell is a cause of increased PG synthesis, and this provides a potential link between increased AR activity and functional and structural changes in tissues susceptible to diabetic complications.

Effect of inhibitors of arachidonic acid metabolism on IGF-1-induced growth of proximal tubular cells in primary culture. *M. Gekle, P.T. Heng, C.A. Pollock, Department of Medicine, Royal North Shore Hospital, St. Leonards, NSW, Australia.* Insulin-like growth factor I (IGF-1) is involved in the regulation of kidney growth during maturation as well as during regenerative processes. One site of action of IGF-1 are cells of the proximal tubular epithelium. The action of IGF-1 is mediated by plasma membrane receptors with kinase activity. Although the subsequent events of intracellular signal transmission in proximal tubular cells have not yet been elucidated in detail, some observations point to eicosanoids as possible mediators. We investigated the effects of different inhibitors of arachidonic acid (AA) metabolism on IGF-1-induced hypertrophy (increase in cell protein) and hyperplasia (increase in cellular thymidine incorporation and cell number) of rat proximal tubular cells in primary culture. All results are expressed as Δ% versus control (mean ± SEM). Forty-eight hour incubation with IGF-1 led to an increase of cellular protein (+36 ± 5%, N = 25), cellular thymidine incorporation (+74 ± 10%, N = 20) and cell number (+27 ± 6%, N = 13). The hypertrophic effect of IGF-1 was abolished completely by 50 μmol/liter dibucaine (inhibitor of phospholipase A_2) or 10 μmol/liter proadifen (inhibitor of cytochrome P-450 enzymes). Ten μmol/liter of clotrimazole (inhibitor of epoxygenase) did not abolish, but reduced the hypertrophic response significantly to +21 ± 4%, N = 13. Indomethacin (10 and 100 μmol/liter, inhibitor of cyclooxygenase) or NDGA (10 and 100 μmol/liter, inhibitor of lipoxygenase) did not reduce IGF-1-induced hypertrophy. The IGF-1-induced increase in thymdine incorporation was abolished completely in the presence of 50 μmol/liter dibucaine and significantly reduced in the presence of 10 μmol/liter proadifen (+20 ± 11%, N = 9) or 10 μmol/liter clotrimazole (+25 ± 13%, N = 10). Indomethacin did not affect the hyperplastic response, whereas NDGA reduced it slightly but significantly to +43 ± 8%, N = 7. We conclude that the action of IGF-1 on growth of proximal tubular cells is, at least in part, mediated by metabolites of arachidonic acid. The differential effects of the inhibitors of AA-metabolism suggest that the cytochrome P-450 products (such as epoxids and HETE) serve as intracellular signals for IGF-1. To a lesser extent products of the lipoxygenase may be involved in the mediation of the hyperplastic effect of IGF-1.

Effects of an insulin-like growth factor-I variant in rats with acute tubular necrosis. *H.M. Healy, C.M. Gillespie, J.C. Wallace, A.A. Martin, CRC for Tissue Growth and Repair, Department of Biochemistry, University of Adelaide, and Child Health Research Institute, North Adelaide, SA, Australia.* Insulin-like growth factor-I (IGF-I) has been shown to enhance the recovery of rats from acute tubular necrosis following an episode of renal ischemia. In this study we administered the LR^3- variant of IGF-I to rats by continuous subcutaneous infusion (1.5 mg/kg body wt/day) for 7 days after a 45 minute period of bilateral clamping of the renal arteries. A similar group of rats was treated with vehicle alone. At the end of the 7 day treatment period, histological examination of the kidneys of the LR^3IGF-I-treated rats demonstrated a significantly greater degree of tubular necrosis than the vehicle-treated animals. Protein and DNA content of the kidney increased in the 7 days of recovery, with no differences between the two treatment groups being apparent. Expression of the protooncogenes c-*myc* and c-*jun* was elevated in the kidney during the early period of recovery (3 days) in vehicle-treated rats, while LR^3IGF-I administration resulted in prolonged expression of both protooncogenes for the full 7 days of observation. Renal angiotensinogen mRNA levels were decreased in both groups at 3 days, but levels increased markedly towards normal at 7 days with administration of the IGF analogue. Renin mRNA was unaffected by either treatment. Plasma renin activity was reduced at 3, but not 7, days in vehicle treated rats, with LR^3IGF-I treatment having no significant effect. Plasma levels of IGF-I were decreased in the analogue-treated rats at 3 days, with hepatic levels of IGF-I mRNA also being reduced at this time. These data suggest a deleterious effect on repair of acute tubular necrosis by LR^3IGF-I administration throughout the recovery period.

Efficacy and renal toxicity of a new ^{23}Na-NMR spectroscopy shift reagent, $TmDOTP^{5-}$. *I.A. Leditschke, G.J. Cowin, D. Willgoss and Z.H. Endre. Renal Research Unit, University of Queensland Department of Medicine, Royal Brisbane Hospital, Herston, Brisbane, Australia.* Transcellular sodium gradients reflect cellular energetics and are a marker of early cellular injury in experimental acute renal failure. ^{23}Na nuclear magnetic resonance (NMR) spectroscopy can monitor these gradients non-invasively on a biologically useful time-scale. However, renal studies have been limited by toxicity of the shift reagents (SR) needed to distinguish the intracellular sodium signal from that of the extracellular compartment. Nephrotoxicity was manifested by increased renal vascular resistance (RVR) and fractional sodium excretion (FE_{Na}) and a reduced inulin clearance. We assessed the new SR, $TmDOTP^{5-}$, in the isolated perfused rat kidney, and demonstrated excellent resolution (~3 ppm) of intra- and extracellular sodium signals at perfusate concentrations as low as 4 mmol/liter. To assess renal toxicity, right kidneys from male Sprague-Dawley rats were perfused with Krebs Henseleit buffer with 6.5% albumin and gassed continuously with 95% O_2/5% CO_2. Perfusion pressure, flow and oxygen tension were continuously monitored. Changes in renal function (inulin clearance, sodium and potassium excretion) were compared in normal controls (N = 4) and the treatment group (N = 5) perfused for 60 minutes prior to the addition of $TmDOTP^{5-}$ to a perfusate concentration of 4 mmol/liter. At 120 minutes kidneys in both groups were freeze clamped for adenine nucleotide assay. There were no significant differences between the two groups (by paired t-test) in inulin clearance, FE_{Na} and FE_K, adenine nucleotides or RVR. We conclude that this is a promising new shift reagent with improved resolution of intra- and extracellular sodium signals, and with no evidence of renal toxicity at a relatively low perfusate concentration. $TmDOTP^{5-}$ should prove very useful in the further elucidation of the role of sodium gradients in renal physiology and pathophysiology.

Parathyroid hormone (PTH) effects on skeletal muscle bioenergetics. *C.H. Thompson, Y.S. Green, G.J. Kemp, G.K. Radda, MRC Biochemical and Clinical Magnetic Resonance Unit, Oxford Radcliffe Hospital, Oxford, England, United Kingdom.* Skeletal muscle cytosolic inorganic phosphate concentration ([Pi]) is relatively insensitive to changes in plasma [Pi] because cell [Pi] is controlled by a Na-Pi co-transporter with a Km *in vitro* of 0.1 to 0.3 mM. PTH has been shown to reduce Pi transport in the kidney and to cause mitochondrial impairment in skeletal muscle *in vitro*. Disordered skeletal muscle Pi homeostasis may underlie the myopathy that occurs in primary hyperparathyroidism or in the secondary hyperparathyroidism of chronic renal failure. We studied Wistar rats (200 g) injected i.p. with either PTH (200 U) or saline for 4 days. ^{31}P magnetic resonance spectroscopy allowed measurement of the concentration of resting skeletal muscle, cytosolic Pi and the rates of oxidative (Q_{max} mmol/kg/min) and anaerobic ATP turnover of the muscle during sciatic nerve stimulation at 2 and 10 Hz and during recovery. PTH caused a 22% reduction in plasma [Pi] and a 40% reduction in intracellular [Pi] (both $P < 0.05$). This disproportionate reduction in cell [Pi] suggests that the Na-Pi co-transporter is down-regulated. Despite this reduction in (metabolically-active) cell [Pi], there was no alteration in muscle acrobic (Q_{max}: 21 ± 2 in PTH-injected cf 19 ± 3 in controls) or anaerobic performance during exercise or recovery. Proton efflux from the muscle (mmol/kg/min) was unchanged (13 ± 5 in PTH-injected cf 18 ± 5 in controls). Reduced mitochondrial function *in vitro* following 4 days of PTH injection is not confirmed *in vivo*. The myopathy of hyperparathyroidism may not be due to changes in cell [Pi] or to elevation of circulating levels of PTH *per se*.

Acute effect of parathyroid hormone (PTH) on urine concentration. *S.L. Carney and A.H.B. Gillies, Department of Medicine, University of Newcastle, NSW 2305, Australia.* Hormones other than arginine vasopressin (AVP) may directly alter distal nephron water transport although their physiological importance is unclear. While PTH has been shown to act as a partial agonist to AVP *in vivo, in vitro* studies with PTH and $PTH_{1-}P$ have been contradictory in experimental animals. Therefore, the following

experiments were performed in anaesthetised water diuretic thyroparathyroidectomized rats to evaluate the effect of PTH on urine concentration, in the presence and absence of AVP. A maximal phosphaturic concentration of rat PTH (2 μg/kg) reduced urine flow from 125 ± 7 to 81 ± 9 μl/min within 10 min ($P < 0.01$). Addition of a maximal antidiuretic concentration of AVP (100 ng/kg) produced a delayed and diminished antidiuretic response when compared to a group of rats not pre-treated with PTH (47 ± 5 compared with 27 ± 5 μl/min, $P < 0.01$). However, a supramaximal AVP concentration (1000 ng/kg) produced a maximal antidiuretic effect in the presence of PTH. In further experiments, a submaximal AVP concentration (7.5 μg/kg) produced a similar antidiuretic effect to 2 ng/kg PTH. The addition of a maximal antidiuretic dose of AVP (100 ng/kg) to both groups was significantly blunted in animals pre-treated with PTH. These studies demonstrate that high physiological concentrations of PTH have a significant direct antidiuretic effect and can also interfere with the action of AVP supporting a partial agonist role.

Acute effect of lithium administration on renal Ca, Mg and PO_4 transport in the rat. *S. Carney and P. Jackson, Department of Medicine, University of Newcastle, NSW 2305, Australia.* Lithium (Li) treatment is known to cause a form of hyperparathyroidism in humans and experimental animals. However, the mechanism for the hypercalcaemia and increased parathyroid hormone (PTH) secretion is unclear, partly due to different experimental protocols. Because a direct effect of Li on PTH induced Ca transport has not been studied, thyroparathyroidectomized (TPTX) and intact rats were studies following the administration of Li. In addition, TPTX Li infused rats were studied following the addition of PTH at maximal phosphaturic doses (2 μg/kg). While Li produced a diuresis and natriuresis in both intact and TPTX rats as well as a phosphaturia, Li also appeared to increase the FE of Ca (2.2 ± 0.2 to 3.7 ± 0.3% $P < 0.01$) and Mg (12 ± 2 to 18 ± 3%; $P < 0.01$) only in intact rats. Li pretreatment blunted the effect of PTH on Ca and Mg reabsorption by approximately 50% ($P < 0.01$). These results suggest that part of the hyperparathyroidism produced by Li treatment is due to an inhibition of the action of PTH within the nephron. A clearer understanding of the mechanism by which Li inhibits the action of PTH may help to define the etiology of primary hyperparathyroidism.

Ganglioside GM2 activator protein gene expression in sheep kidney. *A. Butkus, J. Haralambidis, G.B. Ryan, and J.P. Coghlan, Howard Florey Institute and Department of Anatomy, University of Melbourne Parkville, Victoria, Australia.* Lysosomal glycolipid storage diseases have been recognized for a long time because of their catastrophic neurodegenerative presentation, for example Tay Sachs disease. Three genetically distinct forms of GM2 gangliosidosis, including Tay-Sachs disease, exist. One is the so-called AB variant, where excessive accumulation of the GM2 ganglioside results from a defect in the activator protein. The richest source of this protein is the kidney, then brain and liver; large quantities are found in urine. Much earlier, we had isolated from sheep kidney an enriched granular fraction, which on 2D gel electrophoresis yielded a series of unknown proteins with an apparent molecular weight of 18–22 kDa. Recent sequence comparison of this protein revealed its close homology to the now recognized human GM2 activator protein. As the kidney is a rich source of the activator protein, then the question arises as to whether it is made in the kidney or transported there from other sources and excreted. We used Northern gels and then hybridization histochemistry to demonstrate that the gene encoding the activator protein is expressed in all the major renal arteries and arterioles, and to a lesser extent in the interlobular arteries. Antibodies raised against the GM2 activator protein confirmed that the protein was present at the same sites. These are the first studies showing the location of the GM2 activator gene expression in the mammalian kidney. The arterial site of production has implications for local action and receptor function or an important role in membrane integrity throughout the kidney.

TGF-β type II receptor mRNA in the embryonic rat kidney: An *in situ* hybridization study. *A.T. Clark, M.D. Ford, V. Nurcombe, D. Alcorn, B. Key, and J.F. Bertram, Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, Australia.* The transforming growth factor-βs (TGF-βs) are a family of peptide growth factors that influence cell proliferation, differentiation, extracellular matrix synthesis and turnover, and angiogenesis in many tissues. Recently, TGF-β1 was shown to inhibit tubule branching in the developing rat metanephros. However, the localization of TGF-β receptors in metanephroi remains unknown. The aim of the present investigation was to identify those cells in developing rat metanephroi that express the mRNA for the TGF-β type II receptor, which is specific for the TGF-β family. Metanephroi from embryos of time-mated Sprague-Dawley rats at days 16 (E16), 18 and 20 were fixed in 4% paraformaldehyde and embedded in paraffin. *In situ* hybridization was performed using sense and antisense biotinylated riboprobes. TGF-β type II receptor mRNA was detected in ureteric epithelium, cells of the nephrogenic zone, vesicles, comma-shaped bodies, and the distal portion of S-shaped bodies. However, the mRNA was not detected in the lower limbs of S-shaped bodies, in maturing glomeruli, interstitial cells, or proximal tubules. These results indicate that expression of the TGF-β type II receptor is developmentally regulated. In particular, expression is down-regulated in the lower limb of S-shaped bodies and in glomeruli. A similar down-regulation of expression in the lower limb has recently been described for the DNA binding protein Pax-2. Manipulative studies utilizing both *in vitro* and *in vivo* models are needed to define fully the role of these molecules in metanephric development.

Transforming growth factor β (TGFβ) in compensatory renal hypertrophy (CRH): An electron microprobe study. *C.A. Pollock, M. Nobes, P.T. Heng, A.Z. Gyory, M. Berkahn, A. Ortis, and M.J. Field, Department of Renal Medicine, Royal North Shore Hospital and Electron Microscope Unit, University of Sydney, Sydney, Australia.* Following a reduction in renal mass, immediate adaptive changes occur in glomerular and tubular function, which allow homeostasis to be maintained. Paracrine and autocrine factors are elaborated which initially stimulate kidney growth and later act to limit the growth response. Evidence exists that TGFβ may play a role in limiting the renal tubular proliferative response. The following experiments assessed the effect of TGFβ on cell transport in renal hypertrophy, by measurement of intracellular electrolyte concentrations using electron microprobe analysis (EMPX) in proximal tubular (PT) cells of animals undergoing CRH. Four male Wistar rats underwent right unilateral nephrectomy and simultaneous implantation of an osmotic minipump delivering TGFβ at a rate of 1 ng/hr (UNx TGFβ). Control animals underwent uninephrectomy and infusion of vehicle alone (UNx). One week later animals were anesthetised and infused with Ringers solution at 1.2 ml/100 g/hr. After equilibration, three 30 minute clearance collections were done, after which isotonic RbCl was infused at 0.5 mmol/kg over 30 seconds, as a marker of K transport, and the kidney immediately removed. Clearance data demonstrated a lower GFR in UNx TGFβ compared with UNx animals (0.54 ± 0.07 vs. 1.16 ± 0.31 ml/100 g/min; $P < 0.05$). However, no significant differences were evident in the urine flow (3.3 ± 0.35 vs. 7.2 ± 2.3 μl/min; $P = 0.07$) or fractional excretion of Na (0.07 ± 0.02 vs. 0.16 ± 0.08; $P = 0.2$). EMPX analysis of PT cells revealed a similar $[Na]_i$ in both UNx TGFβ and UNx groups (15.7 ± 0.5 vs. 159 ± 0.5; P = NS). The UNx animals had a high cellular dry weight indicative of cell shrinkage, consistent with previous reports of stimulation of Na,K ATPase in the early post-nephrectomy period. However, the UNx TGFβ animals had a significantly lower dry weight of the PT cells (20.2 ± 0.3 vs. 27.5 ± 0.7; $P < 0.0001$), similar to that seen in two-kidney control animals. Thus, despite a similar $[Na]_i$ in UNx TGFβ and UNx animals, there is a higher absolute amount of intracellular Na in the TGFβ treated animals, consistent with reversal of the stimulated Na,K ATPase in CRH. The hypothesis that TGFβ acts primarily to inhibit basolateral Na,K ATPase activity was confirmed as intracellular Rb accumulation was significantly reduced in the UNxTGFβ animals (5.2 ± 0.3 vs. 6.3 ± 0.4; $P < 0.05$). Thus, the present study indicates that limitation of nephron hyperfunction in CRH may be mediated by TGFβ.

Effect of angiotensin II infusion on plasma erythropoietin in healthy volunteers. *G.B. Pidgeon, R.R. Bailey, K.L. Lynn, A.M. Richards, and M.G. Nicholls, Departments of Nephrology and Cardiology, Christchurch Hospital, Christchurch, New Zealand.* Evidence suggests that angiotensin II (Ang II) may be an important secretagogue of erythropoietin (EPO), both of renal and extrarenal origin. Studies confirming this have not been performed in human subjects. We have tested the plasma EPO response to short-term infusions of both Ang II and noradrenaline (NA) in seven healthy volunteers. In single-blind, randomized, cross-over studies, subjects on a controlled sodium diet were pretreated with either ouabain (Ou) or placebo. On each study day subjects received incremental infusions of Ang II (2, 4, and 8 ng/kg/min, 30 min each dose) and NA (5, 15, and 45 ng/kg/min, 15 min each dose). Mean arterial pressure (MAP) and EPO

responses were measured. Pretreatment with Ou did not affect EPO response and the achieved concentrations of Ang II and NA were similar on each study day. Data from the two study days have therefore been grouped. Mean plasma EPO concentrations increased from 14.4 ± 2.5 mU/ml to 16.4 ± 2.2 mU/ml during the Ang II infusion ($P < 0.05$) and declined after the Ang II infusion to 14.3 ± 2.1 mU/ml ($P = 0.01$). Plasma EPO concentrations did not change significantly during the NA infusion. The mean maximum increase in MAP was 18.4 ± 1.2 mm Hg during the Ang II infusion and 10.5 ± 1.3 mm Hg during the NA infusion. This study confirms that short-term infusion of Ang II at pressor doses leads to a significant increase in circulating EPO concentrations. Further placebo-controlled studies are required to establish the mechanism of this effect and its role in normal physiology.

Interaction between prostaglandins and the renin-aldosterone axis in the natriuresis following saline infusion. *G.S. Stokes, J.C. Monaghan, E.C. Burke, and D.N. Pillai, Hypertension Unit, Royal North Shore Hospital, St. Leonards, and Department of Clinical Chemistry, Prince of Wales Hospital, Randwick, Australia.* Interest in renal effects of prostaglandins has been renewed by reports of reversible oliguria associated with the use of ketorolac, an NSAID widely used in peri-operative pain relief. As NSAIDs inhibit the synthesis of prostaglandins, we investigated the role of prostaglandins, and of other hormones important in renal Na^+ handling, in the first 6 hours after i.v. Na^+ loading. Eleven normal volunteers (8 M, 3 F; mean age 24 ± 2 years) were given an i.v. infusion of 2 liters 0.9% saline from 1000 hours to 1300 hours on two study days more than a week apart. Following Li_2CO_3 500 mg p.o. on the preceding evening, urine was collected every 90 minutes from 0830 to 1600 hours. Placebo or indomethacin 50 mg was administered p.o. at 0745 hours. Renal tubular Na^+ reabsorption was fractionated into proximal and distal components using Li^+, Na^+ and creatinine clearances. On the placebo day only 14 ± 2% of the Na^+ load was excreted in the first 6 hours. Net Na^+ retention was associated with stepwise decreases in plasma albumin concentration, renin activity and aldosterone, whereas urinary dopamine and plasma ANP increased. Indomethacin decreased urinary PGE_2, but produced a distinctly bimodal response in urinary Na^+ excretion, which decreased sharply in 7 subjects (Group A) and was unchanged in 4 (Group B). In Group A, distal tubular fractional Na^+ reabsorption was increased; overall this was highly correlated with the indomethacin-induced change in urinary Na^+ excretion ($r = -0.96$, $P < 0.0001$). In Group B, plasma renin and aldosterone decreased after indomethacin ($P < 0.05$), whereas there was no such effect in Group A. We conclude that prostaglandins may be involved in off-loading Na^+ after i.v. NaCl infusion; whether prostaglandin inhibition interrupts natriuresis appears to depend on the response of the renin-aldosterone axis.

Glomerular number and size following enalapril treatment in postnatal rats. *J.E. McCausland, J.F. Bertram, G.B. Ryan, and D. Alcorn, Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, Australia.* Chronic angiotensin converting enzyme inhibition (ACEI) in maturing rats (weeks 4 to 10) has been shown to retard the growth of glomeruli but to have no effects on renal vascular hemodynamics. Interruption of the renin angiotensin system during early postnatal development in rats has been shown to cause functional and structural renal abnormalities. The purpose of this study was to examine the effect of chronic ACEI on glomeruli during early postnatal development in the rat. Newborn Sprague-Dawley rats were injected with 10 mg/kg/day enalapril ($N = 6$) or saline ($N = 7$) from day 3 to 21. At day 28, rats were euthanased with 60 mg/kg nembutal IP and the kidneys were perfusion fixed with 4% paraformaldehyde for stereological analysis. Using a physical disector/fractionator combination at the light microscopic level, glomerular number and volume were estimated. Enalapril treatment did not alter glomerular number (control 26,780 ± 3,193 (mean ± SD); enalapril 26,299 ± 2,030) or glomerular tuft volume (control 2,974 ± 0.555 × 10^{-4} mm^3, enalapril 3,471 ± 0.714 × 10^{-4} mm^3). In summary, enalapril treatment had no effect on glomerular number, indicating that angiotensin II is not essential for postnatal glomerulogenesis in the rat. The failure to detect any changes in glomerular volume indicates that angiotensin II has different effects on glomerular growth at immature versus mature stages of development.

Renal, cardiac and vascular angiotensin converting enzyme (ACE) in Goldblatt hypertension. *B. Jackson, K. Jandeleit-Dahm, M. Hettiarchichi, D. Paxton, R.B. Perich and C.I. Johnston, Department of Medicine, The University of Melbourne, Austin Hospital, Heidelberg, Victoria, Australia.* The tissue renin-angiotensin system may participate in the regulation of cardiac, vascular, and renal changes associated with hypertension. Tissue ACE is a key component of the tissue renin-angiotensin and is increased in some models of cardiac and vascular hypertrophy. We have undertaken studies in Goldblatt hypertension to dissociate activity in the tissue renin angiotensin from the circulating renin-angiotensin system and to examine the effects of hypertension on kidney, heart, and vessel hypertrophy. Tissue ACE was measured in mesenteric vessels, kidney and hearts of hypertensive rats eight weeks following clipping one renal artery (2K1C) or clipping one renal artery and excision of one kidney (1K1C) ($N = 12$ each). Hypertension was similar in both models. Plasma renin, angiotensin I, and angiotensin II concentrations were elevated in 2K1C, and low or normal in 1K1C compared with shams ($N = 6$/group). Hypertrophy of mesenteric vessels and the heart was similar in both models, however, tissue ACE was increased only in the 1K1C model, where tissue ACE was directly correlated with the degree of hypertrophy ($r = 0.8$, $P < 0.01$). Tissue ACE in the 1K1C rat was unchanged, but was increased in both kidneys of 2K1C rats ($P < 0.05$). Changes of tissue ACE in kidneys, heart and vessels appears to be dependant on the hypertension model, and not an obligatory accompaniment of cardiovascular or renal hypertrophy associated with Goldblatt hypertension.

Effects of an AT_1 antagonist on glomerular size and number in maturing SHR and WKY rats. *M.M. Kett, D. Alcorn, J.F. Bertram, and W.P. Anderson, Dept. of Anatomy and Cell Biology, University of Melbourne, Parkville, and Baker Medical Research Institute, Prahran, Victoria, Australia.* Previous reports have suggested that there is a reduction in glomerular number and changes in glomerular size in the kidneys of the spontaneously hypertensive rat (SHR), indicating a possible glomerular pathogenesis in the development of hypertension. In this study we used newly developed, unbiased stereological techniques to examine glomerular numbers and dimensions in the SHR. The effects of chronic AT_1 blockade on these characteristics were also investigated. Four groups of rats ($N = 6$) were studied: SHR and Wistar-Kyoto rats (WKY) treated with the AT_1 antagonist TCV-116 (3 mg/kg/day p.o.; SHR-T and WKY-T) and SHR and WKY administered the vehicle (gum arabic p.o.; SHR-C and WKY-C). Treatment was from 4 to 10 weeks of age at which stage the kidneys were perfusion fixed at pressures corresponding to the systolic blood pressure (BP) of each rat. Kidneys were then analysed using a physical disector/fractionator combination for estimation of glomerular number and glomerular tuft volume. Systolic BP (mm Hg) was: SHR-C 156 ± 12 (mean ± SD); WKY-C 134 ± 9; SHR-T 137 ± 13; WKY-T 103 ± 10. Two-way ANOVA found no significant effect of either strain or treatment on glomerular numbers: SHR-C 25,075 ± 1,628; WKY-C 25,754 ± 2,749; SHR-T 27,104 ± 4,136; WKY-T 24,447 ± 2,743. Similarly, there was no effect of strain on glomerular tuft volume, however treatment with TCV-116 significantly ($P < 0.05$) reduced glomerular tuft volume; SHR-C 7.24 ± 0.6 × 10^{-4} mm^3; WKY-C 7.18 ± 0.97 × 10^{-4} mm^3; SHR-T 6.51 ± 1.2 × 10^{-4} mm^3 and WKY-T 5.72 ± 1.25 × 10^{-4} mm^3. We found no differences in either glomerular numbers or dimensions between SHR and WKY rats to account for the hypertension seen in the SHR. Furthermore, chronic blockade of the AT_1 receptor resulted in reduced glomerular tuft volume in both SHR and WKY rats supporting a role for Ang II mediated glomerular growth via the AT_1 receptor.

Characterization of a nonpeptide orally active endothelin (ET_A and ET_B) receptor antagonist. *J. Risvanis, P.A. Phillips, K. Aldred, and L.M. Burrell, Department of Medicine, University of Melbourne, Austin Hospital, Victoria, Australia.* Endothelin [(ET)-1] has been shown to play a role in modulating the homeostatic control of blood pressure via its ET_A and ET_B type receptors. We have assessed the *in vivo* and *in vitro* binding characteristics of a novel orally active non peptide ET_A and ET_B receptor antagonist, bosentan (Ro 47-0203), in mesenteric vascular membranes and hepatocyte membranes from Wistar-Kyoto rats (~200 g). Maximum inhibition of [^{125}I]ET-1 binding in mesenteric vascular membranes by bosentan (100 mg/kg) occurred at 1 hr post-dosing returning to control levels within 4 hr. Maximum inhibition of [^{125}I]ET-1 binding in hepatocyte membranes by bosentan occurred at 4 hr post-dosing and persisted to beyond 8 hr. Scatchard analysis of saturation binding in mesenteric vascular and hepatocyte membranes following oral administration of bosentan (100 mg/kg) indicated an increase in the apparent kDa of the

[^{125}I]ET-1 binding sites consistent with receptor binding by bosentan. There was no significant change in endothelin receptor B_{max} in either membrane preparation. In addition, Scatchard analysis of saturation binding in both membrane preparations *in vitro* with bosentan at 10 nM and 100 nM doses demonstrated a dose dependent increase in the apparent kDa of the [^{125}I]ET-1 binding sites. There were no significant differences in endothelin receptor B_{max} in either membrane preparation. Displacement of [^{125}I]ET-1 from endothelin receptors in mesenteric and hepatocyte membranes following coincubation with endothelin analogues resulted in an order of potency for liver of ET-1 = ET-2 > ET-3 > bosentan > IRL 1038 (an ET_B receptor antagonist) > BQ 123 (an ET_A receptor antagonist) and mesenteric vessels of ET-1 > ET-2 > Bosentan > BQ123 > ET-3 > IRL 1038. The respective IC50 of bosentan from liver and mesenteric vascular membranes was (liver: $\sim 2.6 \times 10^{-8}$ M, mesenteric vessels: $\sim 0.2 \times 10^{-7}$ M). Bosentan, a novel, orally active endothelin receptor antagonist is a competitive antagonist both *in vivo* and *in vitro* in the liver and the mesenteric vasculature.

Systemic endothelin in primate pregnancy and preeclampsia. *A. Hennessy, A.G. Gillin, J.S. Horvath, and D.J. Tiller. Department of Renal Medicine, Royal Prince Alfred Hospital, Camperdown NSW, Australia.* An association between increased venous endothelin (ET) concentration and hypertension has been suggested in human pregnancy. The source of this endothelin has not been elucidated. It is postulated that it is produced by the uteroplacental unit in preeclamptic pregnancy. The aim of this study was to examine the uteroplacental circulation in normal baboon pregnancy and experimental preeclampsia for changes in endothelin concentration. Ten baboons were examined serially from 8 weeks gestation (P8) and blood was collected each four weeks (P12, P16, P20 and P24). The same baboons were tested non-pregnant (NP). Blood was collected from the inferior vena cava, aorta and cephalic vein to represent uterine venous effluent, arterial blood and peripheral venous blood respectively. In a separate experiment, blood from these sites was collected in three chronically catheterized animals, two with induced uteroplacental ischemia and evidence of preeclampsia and 1 sham-operated control. ET was measured by radioimmunoassay. There was a slight increase in ET from non-pregnant values to early pregnancy values in venous blood. Arterial blood, however, showed a significant ($P < 0.01$) decrease from non-pregnant values to the end of pregnancy with the lowest concentration recorded in late pregnancy at all collection sites: 1.83 ± 0.46 pmol/liter (NP), 1.49 ± 0.38 (P8), 0.88 ± 0.10 (P12), 0.90 ± 0.19 (P16), 0.92 ± 0.15 (P20) and 0.68 ± 0.15 pmol/liter (P24). There was an increase in ET concentration from all sites in the chronically catheterized animals. The animal with clinical evidence of severe preeclampsia (thrombocytopenia and hypertension) showed a 2.5 × increase in arterial ET after induction of ischemia and no change in uteroplacental ET concentration. This study suggests a systemic role for ET in preeclampsia. Elevation of ET concentration above non-pregnant levels in human pregnancy may be a marker of preeclampsia and aid in diagnosis.

Endothelial dysfunction in cyclosporine A-induced hypertension in rats. *B. Bartholomeusz, P.A. Phillips, K. Rolls, L.-M. Burrell, and K.J. Hardy, Departments of Surgery and Medicine, Austin Hospital, University of Melbourne, Victoria, Australia.* Cyclosporine A (CsA) prevents organ rejection but causes hypertension. CsA damages endothelial cells and may reduce endothelial-derived nitric oxide (NO) release, contributing to the hypertension. CsA increases endothelin-1 (ET-1) synthesis in rats *in vivo* and in human cultured endothelial cells *in vitro*. This study examines the effects of NO enhancement with L-arginine and endothelin blockade with bosentan (Ro 47-0203), an orally active, non peptide, ET_A and ET_B receptor antagonist. Female Wistar rats received CsA (10 mg/kg/day, s.c.) or Vehicle (polyvinylpyrrolidone, 400 μl) for 30 days. Tail cuff systolic blood pressure (SBP) was measured twice weekly. During the last week of CsA treatment, rats received either L-arginine (250 mg/kg/day, in saline, i.p.), saline (0.6 ml), bosentan (100 mg/kg/day in arabic gum, by gavage) or arabic gum (0.5 ml). SBP was measured daily during this last week. At the end of treatment, rats in the NO groups were placed in metabolic cages for 24 hr and rats in the ET group had mean arterial pressure (MAP) measured via a carotid artery cannula. Rats were killed and blood CsA, and plasma creatinine, hormone and electrolyte concentrations were measured. L-arginine lowered SBP in the CsA group (saline 134 ± 2 mm Hg vs. L-arginine 111 ± 6 mm Hg, $P < 0.01$), to the level of the normotensive control rats that received saline. L-arginine lowered SBP in normotensive rats ($P < 0.05$). Bosentan lowered SBP in CsA-hypertensive rats compared to vehicle by day 4 (134 ± 2 vs. 122 ± 3 mm Hg, $P < 0.01$), but had no effect in the normotensive group. The MAP for the CsA-treated rats that received bosentan was lower than that of the rats treated with gum (bosentan 114 ± 10 mm Hg vs. gum 129 ± 9 mm Hg, $P < 0.01$). Therefore, modulation of the endothelium-derived vasoconstrictor, ET-1 and vasodilator, NO lowered blood pressure in CsA-hypertension, suggesting that changes in these two systems may contribute to the development of CsA-induced hypertension.

Renal amylin binding site in rat models of hypertension. *Z. Cao, P.J. Wookey, R. Komers, and M.E. Cooper, Department of Medicine, University of Melbourne, Heidelberg Repatriation Hospital, Heidelberg West, Victoria, Australia.* Amylin, a 37 amino acid peptide, is cosecreted with insulin from the pancreatic β-cells. In the normal rat kidney, we have shown that high affinity (< nM range) binding sites for amylin are associated with proximal tubules in the outer cortex. Furthermore, these sites are functionally significant, since amylin (10^{-9} M) introduced on the basolateral side of the epithelium in micropuncture experiments (shrinking oil drop) stimulated sodium/water resorption by 30%, demonstrating that amylin is equipotent in this regard with angiotensin II or endothelin. Low doses of amylin stimulate plasma renin activity at least twofold in rats and human subjects. These results suggest that amylin may play a role in the development of hypertension, particularly common in type II diabetic and obese subjects, in which amylin levels are elevated. We have explored the relationship between amylin and hypertension, by assessing the high affinity amylin binding site in rat models of hypertension and obesity. Animals were anaesthetized with pentobarbitone (100 mg/kg, i.p.) and nephrectomized by removal of one kidney and occlusion of two of the remaining three branches of the renal artery (5/6th Nx model) or clipping renal artery (Goldblatt model, 2 kidney/1 clip) in 8-week-old Sprague-Dawley rats. Blood pressure was monitored by tail cuff plethysmography every other day, and after 4 weeks when blood pressure had risen to a maximum (> 190 mm), the animals were sacrificed painlessly (12 weeks total age) by a single injection i.p. of nembutal (60 mg/kg). The kidneys were removed, snap frozen in isopentane (−70°C) and 20 μm sections mounted on glass slides for binding studies with [^{125}I]-amylin and [^{125}I] AC512, a peptide analogue of amylin. The net binding (total minus nonspecific binding) was elevated in the outer cortex of the 5/6th Nx model compared to normal kidneys, and this pattern of binding extended into the ischemic region. However, with increasing competition provided by increasing concentrations (10^{-11} to 10^{-6} M) of non-radioactive peptide, binding was reduced to background levels in the healthy outer cortex, but was virtually unaffected in the ischaemic regions, suggesting a change in the pharmacological characteristics. In the Goldblatt model, there was no change in the binding in the contralateral kidney but an apparent increased intensity of binding in the stenosed, ipsilateral kidney. Furthermore, amylin stimulated adenylyl cyclase activity in membranes from normal and the contralateral outer cortex by two- to threefold. In tissue from the Goldblatt ipsilateral kidney, the basal level of adenylyl cyclase activity was ten times that found in the normal or contralateral kidney, and there was no apparent stimulation by amylin. We postulate that amylin is an important hormone in renin regulation and blood pressure control.

Noninvasive diagnosis of nephrosclerosis: Microvascular ischemic nephropathy by renal duplex ultrasound. *S.J. Chadban and R.S. Nanra, Nephrology Unit, John Hunter Hospital, Newcastle, NSW, Australia.* It has been suggested that renal duplex ultrasound (RDU) signals from renal cortex provide an index of cortical blood flow. The aim of this retrospective study was to evaluate the use of the ratio of diastolic to peak systolic Doppler frequency signals (EDRs) obtained from renal cortex in a human clinical model of hypertensive microvascular ischemic nephropathy (HMIN), the contralateral kidney (RAS−) in unilateral significant renal artery stenosis (RAS); the RAS+ protected kidney was used as control. Of 111 patients examined for RAS by RDU and angiography, 89 patients with renal artery thrombosis, bilateral RAS, no RAS, primary renal disease, no EDR values, single kidneys, or absence of hypertension, were excluded from analysis. Thus, 22 hypertensive patients with angiographic unilateral significant RAS (>50%) were analyzed: male:female ratio 1.4:1, mean age 64 years (SD 9), mean C_{Cr} 41 ml/min/1.73 m^2 (SD 13), mean duration of hypertension 12 years (SD 11), mean MAP 121 mm Hg (SD 22), and mean number of antihypertensive drugs 2.3 (SD 1.1). The mean EDR from RAS− kidneys 0.26 (SD 0.09), was lower than the mean EDR from RAS+

controls, 0.33 (SD 0.09) (t = 4.35, $P = 0.0003$). In 9 patients in whom RAS was invasively corrected, the mean pre-procedure EDRs, RAS+ 0.35 (SD 0.07) and RAS− 0.28 (SD 0.07), remained unchanged compared to post-procedure EDRs, RAS+ 0.32 (SD 0.09) and RAS− 0.28 (SD 0.13). An EDR value ≤0.30 detected HMIN with a sensitivity of 77% and specificity of 64%. It is concluded that EDR ≤0.30 is a useful additional test in the diagnosis of HMIN. EDR may also assist in evaluation and management of essential and renovascular hypertension, and in the diagnosis of other forms of nephrosclerosis.

Effect of fluvastatin on lipoprotein profiles in renal transplant patients with dyslipoproteinemia. *P.K.T. Li, T.W.L. Mak, T.H. Chan, A.Y.M. Wang, S.F. Lui, C.W.K. Lam, and K.N. Lai, Department of Medicine and Chemical Pathology, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong.* The objective of this study is to investigate the use of fluvastatin (F), a new HMG CoA reductase inhibitor, in treating dyslipoproteinuria in renal transplant patients. Sixteen renal transplant patients who were on cyclosporine A (CsA) were studied. The patients had elevated plasma cholesterol levels after diet control and required drug treatment. They were studied for a period of 32 weeks with 4 weeks of baseline, 4 weeks of placebo, 12 weeks of treatment with (F) 20 mg and then 12 weeks of treatment with (F) 40 mg consecutively. Blood samples were obtained 4-weekly for the measurements of trough concentrations of CsA, renal and liver functions, creatine phosphokinase (CPK) and lipoprotein profiles which include total cholesterol (TC), triglyceride (TG), LDL-, HDL-, HDL2-, HDL3-, VLDL-cholesterol, apolipoprotein A and B and lipoprotein (a) [Lp(a)]. Significant reductions of lipoprotein levels were observed in TC, LDL-cholesterol and Apolipoprotein B (APB) between the treatment and the baseline period (data as mean ± SEM):

	Baseline	F 20 mg	F 40 mg
TC *mmol/liter*	6.69 ± 0.16	5.81 ± 0.13[a]	5.61 ± 0.14[a]
LDL-C *mmol/liter*	4.1 ± 0.17	3.25 ± 0.11[a]	3.01 ± 0.14[a]
APB *mg/liter*	159.4 ± 4.8	137.8 ± 5.0[a]	132.5 ± 5.5[a]

[a] $P < 0.001$

There was no significant difference in other lipid profiles comparing the baseline and the treatment phases. Serum potassium level was reduced from 4.2 mmol/liter to 4.0 with (F) 40 mg daily. No other significant differences were found in the renal function and liver function tests, CPK and CsA levels in the different periods. No patient complained of myalgia or dropped out from the study due to side effects. Fluvastatin appears to be a safe drug in treating dyslipoproteinemia in renal transplant patients.

Presence and significance of glomerular polymorphonucleocytes in renal transplant insertion biopsy. *P.T.H. Coates, G.R. Russ, and A.E. Seymour, Renal Unit, Flinders Medical Centre, Renal Unit, Queen Elizabeth Hospital and Gribbles Pathology Services, Adelaide, South Australia, Australia.* The presence of glomerular polymorphonucleocytes (PMN) in glomeruli in renal transplant insertion biopsy has been suggested to correlate with graft survival and outcome. We reviewed 189 insertion biopsies to document the number of PMN's and the clinical outcome. A total of 189 insertion biopsies performed in our institution from 1986–1990 inclusive were reviewed by two observers. The number of PMN's on H and E stain in 20 glomeruli were counted and recorded for each patient. Outcome data (serum creatinine and graft failure) was collected from the ANZDATA registry. The average PMN count was 27.5 per 20 glomeruli counted. Patients were divided into 2 groups with PMN count >40 ($N = 36$) and <40 ($N = 163$). In the first twelve months post-renal transplant there were 7 graft failures in the first group and 16 graft failures in the second ($\chi^2 = 2.2$ 1 DF $0.5 < P < 0.1$, NS). Average serum creatinines at 1, 6 and 12 months were 193, 163, 152 and 212, 150 and 152 in the two groups, respectively (NS). In the group with >40 PMN's there was no relationship between absolute PMN count and graft outcome. In conclusion, a high glomerular polymorphonucleocyte count on insertion biopsy does not predict poor graft outcome or early graft loss.

Effect of cyclosporine (CsA) on genetic determinants of factor VIIc (VIIC), fibrinogen (FIB) and cardiovascular disease (CVD) following renal transplantation. *A. Irish and F. Green, Renal Unit, Churchill Hospital, Oxford, and Dept Medicine, UCL Medical School, London, England, United Kingdom.* Thrombotic risk factors Factor VII coagulant activity (fVIIc) and fibrinogen (fib) were measured in 38 stable renal transplant recipients (RTR) and compared with 31 controls (N) matched for age and BMI. Genotypes for the β-fib G/A^{-455} and fVII Arg/Gln_{353} polymorphisms were determined by PCR and restriction enzyme digestion (HaeIII, MspI).

	Fib (mean ± SD) mg/dl	
Genotype	N	RTR
GG	267 ± 46	351 ± 58[a]
GA	270 ± 65	364 ± 54[a]
ArgArg	278 ± 54	372 ± 48[a]
ArgGln	236 ± 39	319 ± 56[a]

	FVIIc % standard	
Genotype	N	RTR
GG	85 ± 21	97 ± 26
GA	85 ± 25	96 ± 35
ArgArg	91 ± 21	106 ± 27[a]
ArgGln	64 ± 6	71 ± 21

[a] $P \leq 0.05$ RTR vs. N

Fib was higher in RTR irrespective of genotype. Fib correlated independently with CsA levels ($r = 0.31$, $P = 0.07$) but this effect was stronger in the A allele ($r = 0.50$) than the G ($r = 0.18$). Interleukin-6 levels were increased in RTR compared with controls (3.6 ± 3.2 vs. 1.9 ± 1.6 pg/ml, $P < 0.05$), suggesting that a persistent acute phase effect contributes to Fib elevation in RTR possibly mediated by CsA. Fib (392 ± 50 vs. 345 ± 54 mg/dl, $P < 0.05$) was higher again in those with CVD. D-Dimer (N: 0.08 ± 0.03 vs. RTR: 0.21 ± 0.31 mg/dl, $P < 0.001$) and F1 + 2 (N: 0.33 ± 0.12 vs. RTR: 0.73 ± 0.57 nM, $P < 0.001$) were also increased in RTR consistent with a hypercoagulable state. However, FVIIc in RTR was significantly higher than N only in the Arg homozygotes and 8/27 (30%) ArgArg individuals compared with only 1/11 (9%) Gln carriers had CVD (P = NS). Mean fVIIc in Gln heterozygotes was around 2/3 that in Arg homozygotes in both RTx and N, consistent with previous observations, and Gln carriers also had lower Fib, F1 + 2 and D-Dimer consistent with a hypocoagulable state. CsA correlated with activated factor XII (XIIa) ($r = 0.68$) only in ArgArg individuals in whom the XIIa/VIIc correlation was strongest ($r = 0.75$ ArgArg vs. 0.49 ArgGln), suggesting that CsA induced activation of fXII may account for the increased VIIc seen in ArgArg RTR compared with N. The β-fibrinogen G/A^{-455} promoter polymorphism is associated with small variation in Fib expression while the fVII Gln_{353} allele with significantly decreased fVIIc and Fib, suggesting that genetic factors influence the expression of the hypercoagulable state and risk of CVD following renal transplantation, perhaps in part mediated by allele specific responses to CsA and other environmental factors.

Quantitation of T cell cytokine mRNA in the sheep model of renal allograft rejection. *G. Patrick, J. Carter, R. Krishnan, M. Rao, and G.R. Russ, Transplantation Immunology Laboratory, The Queen Elizabeth Hospital, Adelaide, SA, Australia.* Tissue expression of cytokine transcripts in allograft rejection may be transient and preceed clinical signs of rejection. This study has quantified the temporal profile of T cell cytokine mRNA expression (IL-2, IL-4, TNFα and IFNγ) in relation to histological evidence of rejection in renal biopsies from unmodified sheep renal allografts. Renal allografts between pairs of alloreactive male Merino sheep were biopsied daily. Samples were snap frozen in liquid nitrogen for cytokine mRNA analysis or fixed in formalin for histological evaluation. The reverse transcription-polymerase chain reaction (RT-PCR) was used to amplify one μg of total RNA in the presence of tritiated dTTP and specific primers for the 4 cytokines. Radiolabelled PCR product was hybridized in liquid phase to a biotinylated oligonucleotide complementary to an internal sequence of the target cDNA and captured by streptavidin-coated microfluor beads. The proximity of tritiated PCR product to the fluor allows it to be detected by scintillation counting with subsequent quantitation by reference to standard curves generated from

PCR-scintillation proximity assay analysis of serial dilutions of plasmids containing ovine cytokine cDNA. Results were obtained from 1 autograft and 6 unmodified allografts. Peak copies of cytokine transcripts ±1 SD (relative to plasmid cDNA) per μg of total RNA are shown in the table with the day of occurrence (in brackets):

	IL-2	IL-4
Autograft	not detected	70.2 ± 14 (no peak)
Allograft 1	1602 ± 104 (3)	1590 ± 46 (3)
Allograft 2	322 ± 68 (5)	1279 ± 372 (7)
Allograft 3	1044 ± 82 (3)	14498 ± 948 (3)
Allograft 4	474 ± 182 (5)	3211 ± 440 (5)
Allograft 5	126 ± 45 (3)	1844 ± 780 (3)
Allograft 6	78 ± 38 (3)	3230 ± 617 (3)

	IFNγ (×1000)	TNFα (×1000)
Autograft	0.4 ± 0.2 (no peak)	5.5 ± 1.3 (5)
Allograft 1	4.4 ± 1.2 (3)	230 ± 29 (1)
Allograft 2	32.6 ± 7.6 (7)	63.5 ± 19 (3)
Allograft 3	32.2 ± 0.5 (7)	5.5 ± 0.9 (5)
Allograft 4	22.8 ± 5.5 (5)	5.6 ± 2.9 (5)
Allograft 5	47.3 ± 6.3 (7)	2.9 ± 0.6 (7)
Allograft 6	3.1 ± 0.2 (5)	1.0 ± 0.3 (5)

Allograft histology showed increasing severity of cellular and vascular rejection from day 2 post-transplantation until graft necrosis at days 8 to 9. In this model, acute renal allograft rejection is associated with a significant increase in mRNA expression for IL-2, IL-4 and IFNγ when compared to the autograft. IL-2 mRNA was detected as early as day 1 in the allografts. Correlation with the histological findings suggests a pivotal role for IL-2 in the rejection process. TNFα mRNA expression suggests that the presence of this cytokine within the graft may relate to the surgical procedure. In conclusion, increased intragraft expression of IL-2, IL-4 and IFNγ message during acute renal allograft rejection suggests that the alloimmune response involves activation of several subsets of functional T lymphocytes.

Low bone mineral density in dialysis patients. *M.S. Stein, D.K. Packham, P.R. Ebeling, J.D. Wark, and G.J. Becker, Departments of Medicine, Diabetes and Endocrinology, Nephrology, The Royal Melbourne Hospital, Melbourne, Victoria, Australia.* Dialysis patients are at risk of low bone mineral density from a combination of factors including 1-α hydroxylase deficiency, hyperparathyroidism and chronic heparin exposure. We assessed the prevalence of low bone mineral density in 160 of our dialysis patients having this measurement as part of their routine clinical management. Bone densitometry was recorded on Hologic QDR1000W and Hologic QDR2000Plus bone densitometers using dual energy X-ray absorptiometry (DXA). Results are expressed as Z scores (standard deviations from age-sex-matched means of a healthy reference population). Patients are routinely scanned at the non-dominant forearm, hip and lumbar spine. This preliminary communication reports results for the hip and forearm. The mean Z score at the ultradistal radius (UD) was −0.98 ($N = 142$). The mean Z score at the femoral neck (FN) was −0.67 ($N = 155$). The prevalence of patients with a Z score worse than −2 at the ultradistal radius and femoral neck was 32 out of 142 (22%) and 23 out of 155 (15%), respectively. Overall, there is a linear correlation between bone density at the forearm and hip sampled within an individual ($r = 0.63$, $P < 0.001$). There is also a correlation in bone density of both compact and cancellous components at the same region: Ultradistal versus 1/3 radius, $r = 0.64$, and trochanteric versus intertrochanteric hip, $r = 0.86$. One hundred and fourteen patients had never received a renal transplant. For these patients Z score was plotted against time on dialysis. In this cross-sectional analysis the time on dialysis was found not to be a strong predictor of Z score. UD versus time $r = -0.12$ and FN versus time $r = 0.14$. Thus ostoepenia is a common problem in the dialysis population and constitutes a significant site-specific increased fracture risk.

Safety and efficacy of enalapril and simvastatin in dialysis patients. *J.F. Collins, R. Robson, S. MacMahon for PERFECT Study Group, Department of Medicine, Auckland Hospital, Auckland, Department of Nephrology, Christchurch, New Zealand.* Dialysis patients have an increased risk of death from cardiovascular disease compared with age-matched normals. Changes in lipoprotein metabolism and alterations in left ventricular (LV) structure and function are common in these patients and likely to contribute to the increased risk. The objectives of this multi-center double-blind, randomized placebo (plac) controlled pilot study were to determine in dialysis patients the effect of simvastatin (simv) on lipids and enalapril (enal) on LV size, to assess the safety of these agents in dialysis patients and to determine whether or not a large scale preventive trial was practicable. Sixty patients on hemodialysis and 47 patients on CAPD were randomized to receive 2 agents in a factorial design (simv, enal; simv, plac; enal, plac; double plac). The initial enalapril dose was 2.5 mg daily titrated up to 5 mg daily or down to 2.5 mg 3/weeks dependent on blood pressure and symptoms, and simv 10 mg daily. Patients had a 2D echocardiogram (echo) and plasma lipid studies (single lab) at baseline and after 6 months of therapy. Hemoglobin, serum urea, electrolytes, albumin, liver enzymes and CK were checked monthly. Analysis was on an intention to treat basis.

	Baseline	
Lipids	Simv	Plac
HDL *mmol/liter*	1.02	1.00
LDL *mmol/liter*	4.14	4.34
Total cholesterol *mmol/liter*	6.25	6.35
	Enal	Plac
BP		
Systolic	138	138
Diastolic	83	84
Hemoglobin *g/liter*	101	105
ECHO		
LVEDD *cm*	5.8	5.7
LVESD *cm*	3.9	4.0
LVMASS *g*	266	263

	6 monthly		
Lipids	Simv	Plac	*P* value
HDL *mmol/liter*	1.02	0.9	0.45
LDL *mmol/liter*	3.12	3.86	0.003
Total cholesterol *mmol/liter*	5.06	5.85	0.001
	Enal	Plac	*P*
BP			
Systolic	131	139	0.20
Diastolic	79	84	
Hemoglobin *g/liter*	97	106	0.23
ECHO			
LVEDD *cm*	5.7	5.6	0.96
LVESD *cm*	4.0	3.9	0.47
LVMASS *g*	254	244	0.72

A total of 30% of patients on the enalapril arm of the trial withdrew because of hypotension. Simvastatin was well tolerated. In conclusion, low dose simvastatin lowers total and LDL cholesterol in dialysis patients and is well tolerated. As enalapril was withdrawn in one third of patients, the possible beneficial effect on LV mass was not confirmed in this study.

Chronic volume expansion in patients with chronic renal failure: Effects on blood pressure and vasoactive hormones. *D.M. Voss, K.L. Lynn, A.L. Buttimore, and E.A. Espiner, Department of Nephrology, Christchurch Hospital and Department of Medicine, Christchurch School of Medicine, Christchurch, New Zealand.* Blood pressure (BP) is critically dependent on sodium status in patients with chronic renal failure. Extracellular fluid volume (ECF) expansion increases BP but the relations between volume change and change in BP and levels of vasoactive hormones are unclear. We studied changes in BP and the plasma hormones ANP, BNP, endothelin (ET), angiotensin II (Ang II) renin (PRA) associated with

changes in ECF in four haemodialysis patients. Patients were studied on regular dialysis (basal state), after 4 days of volume expansion (mean ECF increase 5 liter > dry body wt) and after ultrafiltration. Supine mean BP rose (2.2 to 23%) during volume expansion and fell to baseline at the end of the study. Plasma Ang II and PRA values fell as expected during volume expansion and ET values were unchanged. Before volume expansion plasma levels of ANP and BNP (normal in 1) were raised in most patients. ANP concentrations (34 to 117% above baseline) and BNP concentrations (29 to 85%) rose further with fluid loading in 3 or 4 patients. Patient 4 had elevated ANP and BNP concentrations that did not rise further with ECF expansion. ANP and BNP concentrations returned to near baseline values after ultrafiltration. This study shows that chronic ECF expansion in dialysis patients is associated with an increase in BP and marked elevation in both plasma ANP and BNP without change in ET. In contrast to ANP, increases in plasma BNP greatly exceeded those observed by us in previous studies employing short-term (2 hr) periods of ECF expansion. The biological relevance of these hormones changes remains to be clarified.

L-cysteine improves growth of human peritoneal mesothelial cells (HPMC) *in vitro*. *S.D. Bird, A. Knight, M. Legge, and R.J. Walker, Department of Medicine and Department of Biochemistry, Otago Medical School, Dunedin, New Zealand.* The effect of L-cysteine (Cys) and epidermal growth factor (EGF) added individually to the culture medium (M199) on HPMCs, was investigated. HPMCs were obtained from omentum by enzymatic digestion and grown for one passage. Cells were subpassaged and seeded into 24-well plates with M199 medium, 2.0% FBS and L-glutamine plus the growth agents. Cell growth was measured by the determination of total cell protein ($\mu g \cdot well^{-1}$) and cell counts. Morphology was assessed with phase contrast light microscopy and scanning electron microscopy. Intracellular redox status was determined of by HPLC. Results are mean ± SEM, $N = 4$ omentum samples.

Time (d)	Day 3 Total cell protein	Day 9 Total cell protein
M199 control	24.6 ± 2.0	43.9 ± 2.4
Cys	40.5 ± 4.4[a]	101.0 ± 1.5[b]
EGF	28.7 ± 1.9	65.3 ± 1.0[a]

[a] $P < 0.05$, [b] $P < 0.01$

HPMC exposed to Cys (30 $\mu g/ml^{-1}$), exhibited significantly improved attachment and growth. Attached cells appeared flat and well spread out shortly after seeding and produced a tight polygonal monolayer in 14 days in contrast cells grown in M199 control medium which failed to reach confluence. After an initial lag in cell growth, EGF (0.01 $\mu g/ml^{-1}$) increased cell growth greater than that produced by Cys, however, this was associated with significant changes in HPMC morphology. This study shows that Cys improves the cellular redox status, giving rise to improved cell attachment, growth and morphology of HPMC *in vitro*, which may have clinical relevance in the development of new dialysis solutions.

Determinants of CAPD peritonitis rates in Aboriginal and non-Aboriginal patients. *J. Feutrill, L. Thomas, J. Lazberger, V. Burke, J. Whishaw, D. Carruthers, R. Offer, G. Rangan, A. Parnham, G. Thatcher, and M. Thomas, Faculty of Medicine, University of Western Australia, University Department of Medicine and Department of Nephrology, Royal Perth Hospital, Perth, Australia.* Data from 1989–1992 from our Unit had shown a higher rate of peritonitis in aboriginal (Ab) vs. non-aboriginal (non-Ab) CAPD patients. To determine whether this was confounded by factors other than race *per se*, results for all patients treated for >1 month on CAPD between 1.1.92 and 30.6.94 were analyzed. Patients were allocated to either disconnect (Freeline Solo with K shield, or Y-set), or a long-line system (with UV sterilizing devices) according to pre-dialysis assessment. Demographic, medical, socio-economic and CAPD training/technique data were collected prospectively from ANZDATA and Unit records. Socio-economic status (SES) was determined by postcode (Aust Bureau of Statistics) for metropolitan patients. The means of two CAPD nurses' independent rating of current technique adequacy, understanding, compliance, personal hygiene, motivation, domestic support and home environment were calculated. Chi-square and *t*-test were used for comparisons of categorical and continuous variables, respectively. Thirty Ab and 76 non-Ab patients were treated, with Ab patients being younger (47 vs. 59 years), having a higher proportion of females (60% vs. 34%) and diabetics (53% vs. 21%) and fewer not speaking English as a primary language (37 vs. 10%). Disconnect systems were used in Ab patients more frequently (97% vs. 75%). After 5.5 ± 2.7 days training (Ab = non-Ab), CAPD was performed for 13.7 ± 8.7 months in the study and 22.7 ± 19.1 months in total, with Ab patients showing significantly poorer socio-economic status, technique and psycho-social factors than non-Ab patients. Peritonitis rates were not different between Ab and non-Ab patients in this study (1.2 vs. 1.0 episodes/year), but were greater in patients aged over 50 years (1.3 vs. 0.6), not using a disconnect system (2.1 vs. 0.8), having poor technique (2.1 vs. 0.7), performance (3.9 vs. 0.8), compliance (2.2 vs. 0.7) or motivation (2.7 vs. 0.7). After adjustment for age, sex, SES, diabetes and the use of a disconnect system, only rating of performance and motivation remained significant predictors of peritonitis. CAPD peritonitis rates are determined by individual factors independent of race, with disconnect systems offering a significant protective benefit for those at risk.

Peritoneal catheters—Swan neck versus straight. Is there an advantage? *J. Collins, G. Gamble, and J. Leary for the NZ CAPD Registry, Renal Unit, Auckland Hospital, Auckland, New Zealand.* The New Zealand (NZ) CAPD registry has collected comprehensive data on all NZ patients on CAPD since 1986 (6 centers). A total of 487 new patients commenced CAPD in NZ between October 1990 and November 1994 in whom the first peritoneal catheter type was recorded. The two principle peritoneal catheter types utilized (double cuff straight Tenkhoff, double cuff swan neck) were compared in this analysis. Thirty-nine patients received an alternative catheter. Patient death or transplant was not considered to have caused catheter failure.

	Double Cuff Straight	Double Cuff Swan
N	217	231
Age, median *range*	52 (4–79)	49 (10–74)
Sex *% male*	53%	57%
Connection system *% disconnect*	44%	52%
Race *% Polynesian or Maori*	44%	53%
Median years of catheter survival ± SD	1.6 ± 0.7	1.3 ± 0.7
Median years to first episode of peritonitis ± SD	0.8 ± 0.4	0.9 ± 0.5
Peritonitis rate *months per episode*	16.2	12.3
Migration rate *episodes/100 patient years*	10.8	7.1

$P > 0.05$

The causes of catheter failure were peritonitis (47%), catheter dysfunction or leak (19%), exit site and or tunnel infection (11%), catheter migration (5%) and other (18%). There were no significant differences between these causes and catheter type. Despite comparable groups there were no differences in catheter migration rate, time to first episode of peritonitis, peritonitis rate or catheter survival. The sample, although not randomized has 85% power to detect a difference of 6 months in catheter survival ($\alpha = 0.05$). In conclusion, these results suggest that double-cuff swan neck catheters offer no advantage over double-cuff straight catheters.

Sclerosing peritonitis developing after the cessation of continuous ambulatory peritoneal dialysis (CAPD). *S.J. Coulshed, L.S. Ibels, S.D. Roger, and C.A. Macadam, Department of Renal Medicine, Royal North Shore Hospital, St. Leonards, NSW, Australia.* Sclerosing peritonitis (SP) is a rare but devastating complication of CAPD, and has been seen in 3 of 240 CAPD patients at this hospital (incidence 1.25%). We report the case of a 38-year-old man on CAPD for 9 years which was complicated by recurrent exit site infections, peritonitis and then SP. The patient first presented with hemoperitoneum in April 1993. He had recurrent episodes of peritonitis until October 1993 when the catheter was removed due to loss of ultrafiltration. In July 1994 he presented with 4 months of vomiting and marked weight loss. Investigations showed a partial small bowel obstruction with many multiloculated areas of fluid within the peritoneal cavity, extensive vascular calcification, calcification of the peritoneal lining and bowel and a thickened bowel wall. At laparotomy the entire small

bowel was encased in a thick layer of inflammatory tissue up to 0.5 cm thick. The post-operative course was complicated by an ileus, malnutrition requiring prolonged TPN and repeated drainage of abscesses. The patient was discharged malnourished at his request and has since had repeated admissions for bowel obstructions, abdominal collections and cutaneous fistulae. He will not accept home TPN. This case demonstrates the devastating consequences SP that have not been improved by catheter removal. It was associated with prior repeated episodes of exit site infections, peritonitis, and poor compliance. Dialysis is adequate by hemodialysis, however, malabsorption, repeated vomiting and recurrent abscesses continue.

Experience with polyurethane (Thoratec®) vascular access grafts for hemodialysis. *G. Passaris, E. Savdie, J.M. Hayes, J. Crozier, A. Meek, A. Graham, R.S.A. Lord, and C. Turner, Departments of Renal Medicine and Vascular Surgery, St. Vincent's Hospital, Darlinghurst, NSW, Australia.* A new synthetic vascular access device made of polyurethane, the Thoratec® VAG graft (P.U.G.), has been proposed by its manufacturer as an advance on existing devices due to its sealing properties, which are said to enable cannulation after insertion. To evaluate the device, it was used routinely in all hemodialysis patients requiring A-V access, but unsuitable for native A-V fistula formation, between 31/03/1994 and 30/11/1994. Seventeen devices were placed in sixteen patients (8 male, 8 female; mean patient age 58.9 years) by four surgeons. All surgeons felt the use of a tunnelling instrument was essential because of the high compliance of the P.U.G. At surgery, heparin and antibiotics (cephalosporins or vancomycin ± aminoglycosides) were used in all patients. Eight grafts were functional after 3 months. During this time, four grafts were lost (2 because of recurrent thrombosis; and 1 each removed because of infection and vascular "steal"). The actuarial three month graft survival was 72.4%. Thirteen grafts were successfully cannulated within four days of operation and thereafter. Cannulation was delayed for 6, 7, 31, and 42 days in four instances because of edema and/or hematoma. Eleven thrombotic episodes were observed in five patients; nine of these were successfully treated by thrombectomy. In two cases, however, thrombosis resulted in loss of graft. Of ten patients with previous access device thrombosis, four had subsequent P.U.G. thrombosis. This compared with P.U.G. thrombosis in one of six patients without previous access device thrombosis. Nursing staff and patients reported that P.U.G. was easier to cannulate and bled less after decannulation than polytetrafluroethylene (Goretex®) grafts. Despite the uncontrolled nature of this study the data: (i) confirm that in most cases the P.U.G. can be cannulated within days of placement, dispensing with the need for temporary vascular catheters in many cases; (ii) suggest a device survival for P.U.G. similar to Goretex®; (iii) indicate that polyurethane has very acceptable physical characteristics for cannulation and decannulation.

Endovascular procedure in the maintenance of arteriovenous fistula patency. *R. Awardt, G. Fitt, and A.K. Roberts, Renal Unit and Vascular Units, Austin Hospital, Heidelberg, Australia.* Endovascular procedures such as angioplasty and intravascular stent insertion are increasingly being used to treat arteriovenous fistula stenoses. Surgery may often be avoided and distal veins can be preserved. In this study we have reviewed our experience with these procedures to determine whether or not surgical intervention can be successfully postponed for a significant period. Twenty-four procedures were performed on 13 patients over a period of 2 years. All patients were followed for at least 6 months. Patients were selected after intravenous venography if the lesions were suitable (that is, if the lesion was short or of a proximal vein where surgical access would be difficult). An intravascular stent was inserted if there was significant recoil on post-dilation films. Ten of the dilations were of proximal veins and four included stent placement. There was an initial success rate of 75% (18 of 24). The primary patency rate (defined as the percentage of fistulae patent without any further intervention) after 15 months was 28.4%. The secondary patency rate (defined as the percentage of fistulae patent without any *surgical* intervention, that is, endovascular procedures were allowed) after 15 months was 46.1%. These patency rates compare well with other published series, and suggest that repeated endovascular procedures of arteriovenous fistulae can significantly delay the need for surgical revision and assist in prolonging fistulae life.

Use of a dialysate proportioner to calculate urea clearance. *M.B. Fraenkel, and J.K. Dawborn, Renal Unit, Austin Hospital, Heidelberg, Victoria, Australia.* There has been increasing emphasis on the importance of regular assessment of dialysis efficiency. We have compared three formulae for calculating Kt/V (all of which can be processed using a pocket calculator) to Kt/V derived from direct quantitation of urea removed on hemodialysis. A "dialysis proportioner" was used to collect 1/25 of total dialysis effluent obtained during a four-hour mid-week hemodialysis. The volume of effluent was measured and urea concentration determined. Pre- and post-dialysis body weights were recorded. Formulae for calculating Kt/V were (1) $W/5 + 2.5W\ [(U_1 - U_2)/t]$, (2) $1.18 \times -\ln(R)$, (3) $2.2 - 3.3 \times (R - 0.03 - Uf/w)$ where (W = dry weight, U_1 = pre-dialysis urea, U_2 = post-dialysis urea, t = time between dialyses, R = urea reduction ratio, Uf = volume removed on dialysis. Urea clearance (4) was calculated using V/U (V = total body water × mean plasma urea during dialysis, U = total urea removed). Results in five patients have been analyzed to date. There was no difference between Kt/V calculated using (2) and (3) presumably because no patient had weight loss of > 2.5 kg. There was close agreement in derived Kt/V using (1) and (4). In two patients Kt/V derived using (1) and (4) were sufficiently different from (2) and (3) to have influenced management. In conclusion, use of the dialysis proportioner enables direct quantitation of solutes (including urea) in dialysis effluent. Direct comparison between different dialysis regimes will also be possible.

Continuous veno-venous hemodiafiltration (CVVHD) using bicarbonate-containing dialysate in critically ill children. *P.H. Henning, D. Roy, R.J. Hogg, P. Wilby, and K.F. Jureidini. Department of Nephrology, Women's & Children's Hospital, North Adelaide, South Australia.* To avoid the risk of lactic acidosis when using lactate buffered dialysate for continuous arteriovenous or veno-venous hemodiafiltration in patients (CAVHD or CVVHD) with hepatic and renal dysfunction, we have used bicarbonate buffered solutions. We report our experience with 11 children treated in this manner between 1988 and 1994. The mean age was 4.5 ± 5.5 years (range 3 days to 14 years). Access was via dual-lumen external jugular or femoral vein catheters. Hemofilters were chosen on the basis of patient size and dialysis requirements. Bicarbonate buffered dialysis solution was prepared in a novel fashion. Shortly before use supplementation of a specially prepared base solution with commercially available electrolyte solutions was used to achieve the desired electrolyte concentrations. The mean ultrafiltration rate was 41.4 ± 35 ml/hr. Urea and creatinine clearances were 15.3 ± 6.6 and 16.4 ± 8.62 ml/min, respectively. Metabolic acidosis was readily controlled in all cases. Seven of the 11 patients ultimately recovered normal renal functions.

Influence of volume status on echocardiographic findings in hemodialysis patients. *P.G. Kerr, J. Gelman, and K.C. Wong, with the technical assistance of L. Donelan, Departments of Nephrology and Cardiology, Monash Medical Centre, Clayton, Victoria, Australia.* Echocardiographic (echo) assessment of the cardiac status of hemodialysis (HD) patients has become a frequently used tool, in part due to the high incidence of coexisting hypertension and vascular disease. The question addressed by this study was whether the fluid shifts experienced during HD sessions significantly influences the echo findings. Ten stable HD patients, mean age 47 ± 5 years (range 20–64), were randomly selected for study without bias to patients with known cardiac disease. Transthoracic echo studies were performed immediately prior to and after a standard dialysis session. Standard echo parameters were assessed at each time point and the weight loss achieved in the dialysis session was recorded. The results revealed a significant reduction in chamber sizes following HD with a mean loss of 1.5 ± 0.3 kg: the LVED diameter fell from 5.02 ± 0.20 to 4.60 ± 0.16 cm ($P < 0.05$), the LVES diameter from 2.84 ± 0.22 to 2.47 ± 0.22 cm ($P < 0.05$), and the LA size from 4.29 ± 0.23 to 3.83 ± 0.22 cm ($P < 0.05$). Fractional shortening and wall thickness did not change. The isovolumetric relaxation time and deceleration times were also unaltered by the fluid shifts. On the other hand, the early diastolic filling velocity was significantly altered by dialysis, being 0.73 ± 0.05 m/sec pre-HD and 0.56 ± 0.04 m/sec post-HD ($P < 0.005$), whereas the late diastolic (atrial) filling velocity remained unchanged—0.82 ± 0.05 m/sec pre-HD vs. 0.74 ± 0.07 m/sec post-HD (P = NS). The early:late filling velocity ratio was low at both time points, more so post-HD—0.91 ± 0.06 pre-HD vs. 0.82 ± 0.09 post-HD ($P < 0.05$). This latter ratio indicates impaired diastolic filling, masked to a degree by volume overload pre-HD. There was no correlation between any of the echo parameters and the weight loss achieved. These findings suggest that although fluid shifts during HD do not grossly

influence echo findings, comparative studies in a given patient should be performed at the same point in the dialysis cycle. In addition, diastolic filling impairment was common (8/10 patients post-HD) and this finding may be masked by volume overload.

T cell depletion reduces augmentation of glomerular tissue factor and glomerular fibrin deposition in crescentic glomerulonephritis. *J.H. Erlich, P.G. Tipping, and S.R. Holdsworth, Centre for Inflammatory Diseases, Monash University Department of Medicine, Monash Medical Center, Clayton, Victoria, Australia.* Augmented glomerular tissue factor (TF) activity is associated with glomerular infiltration of macrophages, T cells and prominent fibrin deposition in human and experimental crescentic glomerulonephritis (GN). Macrophages are responsible for this augmented TF and its association with glomerular T cell infiltration suggests that sensitized T cells may play an important role in augmenting macrophage TF expression via a DTH like mechanism. Although T cells may direct up-regulation of monocyte TF *in vitro*, their role in activating glomerular macrophages and augmenting TF *in vivo* is uncertain. The role of T cells in inducing glomerular fibrin deposition (GFD) and glomerular TF was assessed in crescentic GN, induced in presensitised rabbits by i.v. injection of 25 mg/kg of horse anti-rabbit glomerular basement membrane globulin. Rabbits were treated with either a monoclonal anti-rabbit CD5 or an irrelevant monoclonal control antibody (50 mg/kg) one hour prior to anti-GBM globulin, followed by a daily dose of 20 mg/kg. Depletion of circulating T cells was assessed by flow cytometry of peripheral blood and tissue depletion was assessed by immunostaining of the kidney and spleen. T cell accumulation in glomeruli was prevented by treatment with anti-CD5 antibody. On day 4 after anti-GBM globulin, glomerular TF activity, assessed in a one stage prothrombin assay, was significantly reduced (anti-CD5 treated 101 ± 16 mU/10^3 glomeruli, control 202 ± 17 mU/10^3 glomeruli, $P = 0.0015$). GFD, assessed semiquantitatively by immunofluorescence (0 to 3+), was also significantly reduced, although this effect was relatively modest (anti-CD5 treated 1.49 ± 0.04, control 1.75 ± 0.06, $P = 0.003$). These data demonstrate that T cells significantly augment glomerular TF expression in crescentic GN, providing evidence for T cell directed macrophage activation akin to DTH in this disease. The modest reduction in GFD suggests that either a profound inhibition of TF expression is necessary to prevent fibrin deposition or other procoagulant or fibrinolytic molecules such as PAI-1, not directly under T cell control, play an important role in controlling GFD.

Expression of human CD59 by transgenic mice. *C.A. Somerville, B. Van Denderen, J. Allison, M. Pearse, and A.J.F. d'Apice, Department of Clinical Immunology, St. Vincent's Hospital, Victoria Parade, Fitzroy, and The Walter and Eliza Hall Institute, Parkville, Victoria, Australia.* Xenotransplantation would provide a solution to the ever worsening shortage of donor organs for transplantation. However, its clinical application is prevented by the invariable occurrence of hyperacute rejection. Species restriction of the membrane bound complement regulatory factors (CRF), which include CD59, may account in part for this phenomenon. This study was undertaken to examine the effect of expression of human CD59 in xenogenic cells and organs on their susceptibility to attack by human complement. Transgenic mice were created by microinjection of a construct in which CD59 cDNA is under control of the H-2K^b promotor. Transgenic mice were identified by Southern blot of genomic DNA. Using flow cytometry two mice have been demonstrated to express human CD59 on peripheral blood leukocytes, at 30% and 50% of the level on human peripheral blood leukocytes. An immunohistological tissue survey has demonstrated CD59 expression on renal endothelium. Cytotoxicity assays have shown that murine splenic leukocytes expressing human CD59 are partially protected from lysis mediated by human serum and the level of protection appears to be related to the level of expression of the transgene. However, in a Langendorf *ex vivo* heart perfusion model, no significant protection could be shown of transgenic hearts compared to non-transgenic hearts exposed to human serum. An immunohistochemical study of the rejected hearts is underway. Mice expressing the CRF CD55 in addition to CD59 have been created and will be analyzed in an identical fashion. In conclusion, although CD59 affords significant protection of murine cells against human complement, it is likely that additional CRF's will be needed to avert the hyperacute rejection of xenotransplantation.

Antibodies to endothelial cells (AECA) and epithelial cells (AEpCA) in renal transplant recipients. *L.M. Johnstone, R.G. Walker, and G.J. Becker, Department of Nephrology, Royal Melbourne Hospital, Parkville, Victoria, Australia.* AECA have been described in different diseases affecting the renal system, including renal transplantation (Tx). Their significance is unclear, although it has been proposed that acute graft rejection may be due to AECA. Rapid onset acute graft rejection has also been attributed to AEpCA. The presence of AECA and AEpCA was investigated in 2 groups of patients: (i) Tx recipients with stable renal function compared with controls (C); (ii) in sera from 11 patients who received renal transplants studied at the time of an episode of acute rejection and compared with pre-transplant sera. AECA and AEpCA were detected using a cellular ELISA; AECA were tested against human unfixed umbilical vein endothelial cells and AEpCA were tested against monkey kidney epithelial cells (CSL) commercial cell line. Binding of cells was exposed with goat anti-human immunoglobulin (IgG) and goat human total immunoglobulin (Ig) with an alkaline phosphatase label. All sera were tested in triplicate and ELISA ratio (ER) was calculated for each set of sera, that is, ER (%) = [(sample OD − negative control OD)/(positive control OD − negative control OD)] × 100. Results:

		IgG %	Total Ig %
AECA	C ($N = 22$)	10.4 (1.4–37.0)	17.1 (5.3–48.2)
	Tx ($N = 53$)	16.9 (2.5–87.0)	26.9 (0–75.9)
AEpCA	C ($N = 22$)	22.3 (0.0–88.8)	29.0 (0.0–90.6)
	Tx ($N = 41$)	19.9 (0.0–74.6)	61.8 (0.0–369.0)

Median (range) $P > 0.05$. Wilcoxon rank-sum test compared to C.

Eleven subjects studied at the time of an episode of acute biopsy proven rejection and compared with pre-transplant levels showed no difference between the pre-transplant ER and the rejection associated ER for both IgG and total Ig for AECA and AEpCA (Wilcoxon matched paired test, data not shown). It was concluded that both AECA and AEpCA did not appear to play a role in acute allograft rejection and were not increased in the general Tx population compared to C.

Gal alpha 1,3 Gal: The dominant xenoantigen. Its detection and role in porcine and murine models of xenotransplation. *M. Tange, Z. Zannettino, A.M. Fournier, M.J. Pearse, R.J. Crawford, and A.J.F. d'Apice, Immunology Research Centre, St. Vincent's Hospital, Fiztroy, Melbourne, and Bresatec Ltd., Thebarton, Adelaide, Australia.* Anti-Gal antibody, which binds to terminal alpha 1,3 galactose (Gal), has previously been demonstrated to bind to porcine organs and endothelium. It has a functional role in xenotransplantation demonstrated in cytotoxicity assays of porcine aortic endothelial cells (PAE) and in *ex vivo* models of xenotransplantation. IB_4 lectin also binds to alpha 1,3 methyl galactopyranosyl sugars. A competition study of IB4 lectin and anti-Gal antibody binding to PAE cells was undertaken. Analysis of binding was determined by FACS. IB4 lectin and anti-Gal antibody both bind to PAE cells, but neither inhibits the binding of the other, suggesting that they each bind at a different site on the Gal epitope. Depletion of anti-Gal antibody was achieved by absorption of human serum (NHS) on COS cells transfected with alpha 1,3 galactosyltransferase. COS cells, which are derived from an Old World monkey, do not express Gal. This gene and a defective construct were transfected by DEAE in a transient expression system. FACS analysis with IB4 lectin demonstrated Gal expression in COS cells transfected with alpha 1,3 galactosyltransferase. There was no expression in the cells transfected with the defective construct. NHS was absorbed sequentially on 2 flasks of transfected COS cells. Following absorption of the NHS there was a 72% decrease in IgG NHS binding to the pig kidney epithelial cell line (PK1) and a 50% decrease in IgM NHS binding to PK1 cells. This supports the role of anti-Gal as a major xenoantibody. The most effective means of removal of alpha 1,3 Gal as a xenoantigen will be by a gene knockout. The 129 SV mouse is an animal in which this technology is currently available. A histological survey of IB4 lectin binding in 129 SV mice was undertaken. There is staining in the endothelium of all organs and in the glomeruli of the kidney, in the bile ductules in the liver and in a perinuclear pattern in the heart. Mouse lung stains strongly. This establishes the presence of alpha 1,3 Gal.

The effects of chronic ouabain infusion in sheep. *G.B. Pidgeon, A.M. Richards, M.G. Nicholls, R.R. Bailey, K.L. Lynn, L.K. Lewis, and T.G. Yandle, Departments of Nephrology, Cardiology and Endocrinology,*

Christchurch Hospital, Christchurch, New Zealand. To examine the contribution of ouabain (Ou) to blood pressure control, we have studied the haemodynamic, renal and hormonal effects of chronic Ou infusion in 8 Coopworth sheep on a controlled sodium diet. Placebo (5% dextrose) was infused for 6 days, followed by Ou (0.25 mg/day) for 22 days, and then a further 7 days of placebo infusion. Blood pressure (BP) and heart rate (HR) were measured and 24 hr urine collections made daily. Weekly 24 hr BP & HR recordings were made. The pressor response to infused angiotensin II (AII) was studied prior to, during, and after the Ou infusion. Plasma concentrations of Ou and other vasoactive hormones were measured. Mean Ou concentrations were greater than 1 nmol/l throughout the infusion period but undetectable prior to commencing the infusion. BP was unchanged by the Ou infusion, but HR was significantly reduced during the first ten days of Ou infusion ($P < 0.03$). Mean 24 hr BP was lower in the third week of Ou compared to either pre- or post-Ou ($P < 0.05$), and mean 24 hr HR was lower in the first week of infusion ($P < 0.02$). The pressor response to AII was similar in all phases of the study. Daily urine volume fell below pre-Ou values in the first week of Ou ($P < 0.01$), and sodium excretion declined over the first ten days of infusion ($P < 0.05$). Mean plasma renin activity & AII concentrations decreased after one week of Ou ($P < 0.05$), but aldosterone was increased ($P = 0.05$) as was cortisol ($P < 0.05$). This study demonstrates, in sheep, that chronic infusion of Ou does not cause hypertension nor alter pressor responsiveness, and it is not natriuretic. This study also fails to confirm reports that Ou is an endogenous hormone.

A randomized, controlled trial comparing 1.25 mmol/liter calcium (Ca) dialysate to 1.75 mmol/liter Ca dialysate in CAPD patients. *D.W. Johnson, R.J. Rigby, H.D. McIntyre, A. Brown, and J. Freeman, Department of Nephrology, Princess Alexandra Hospital, Brisbane, Queensland, Australia.* Effective control of hyperphosphatemia and hyperparathyroidism in CAPD patients requires a combination of calcitriol and $CaCO_3$, but is frequently limited by hypercalcemia. Reducing dialysate Ca concentration may overcome this problem, but this has not been examined in a controlled trial. Forty-five stable CAPD patients were randomly assigned in a prospective, double-blind trial to either a study group (1.25 mmol/liter Ca dialysate) or a control group (1.75 mmol/liter Ca dialysate) for 12 months. Twenty-three patients did not complete the study due to death (9), transplantation (7), or conversion to hemodialysis (7). Eleven patients in each group completed the study. Mean serum Ca, phosphate, ionized Ca, aluminum, alkaline phosphatase and bone mineral density Z-scores did not differ significantly at any time within or between the two groups. Severe hypercalcemia was more common in the control group (11 vs. 2, $P = 0.027$). Mean serum PTH and osteocalcin initially rose in the study group, relative to controls at 3 months (40 ± 7 vs. 12 ± 3 pmol/liter, $P = 0.004$, and 33 ± 5 vs. 15 ± 2 μg/liter, $P = 0.002$, respectively), but was not sustained. Median weekly dosages of calcitriol and daily dosages of $CaCO_3$ increased significantly in the study group (0 μg to 1 μg, $P = 0.014$ and 1260 g to 2520 g, $P = 0.002$, respectively), but not in controls. Supplementary $Al(OH)_3$ was required for phosphate control in both study ($N = 5$) and control patients ($N = 4$). In conclusion, lowering dialysate Ca concentration reduces the frequency of severe hypercalcemia and allows prescription of larger quantities of calcitriol and $CaCO_3$. However, it may potentially initially exacerbate secondary hyperparathyroidism. In this study, there was no effect on bone mineral density or on $Al(OH)_3$ intake.

Validation of Banff histological criteria for acute renal allograft rejection. *W. Hongwei, S.J. Chadban, R. Murugasu, A. Price, and R.S. Nanra, Nephrology Unit and Division of Anatomical Pathology, John Hunter Hospital, Newcastle, New South Wales, Australia.* To validate the Banff histological criteria for acute rejection, a retrospective study was undertaken in 52 graft biopsies from 37 patients [mean age 43 years (SD 13) and female:male ratio 1.2:1] taken during acute rejection episodes (AREs). All AREs were confirmed by: (1) acute rise in mean S_{Cr} from 171 (SD 85) to 338 (SD 268) μmol/liter; (2) histological evidence of acute rejection by standard criteria in adequate biopsies (≥7 glomeruli and ≥1 arteriole/section); and (3) decline in mean S_{Cr} to 141 (SD 83) μmol/liter with antirejection therapy, or graft loss with refractory rejection (7 cases). Original standard grades rejection are as tabulated; all slides were masked and graded independently by 4 blinded observers using the Banff criteria:

Banff rejection grade	Observer 1	2	3	4	Standard rejection grade
Borderline	19	21	18	22	29 (Mild)
I	21	16	23	18	13 (Moderate)
II	8	9	6	7	4 (Severe)
III	4	6	5	5	6 (Vascular)

There were no significant differences between observers ($P = 0.85$) and between Banff and standard rejection grades ($P = 0.24$). By Banff criteria 38% of AREs would receive no antirejection treatment compared to 0% using standard criteria ($P < 0.001$). It is concluded that Banff criteria for acute rejection are reproducible, but may lead to undertreatment of mild rejection episodes.

The pathogenetic mechanism of hepatitis B virus (HBV)-related glomerulonephritis. *K.N. Lai, R.T.H. Ho, P. Li, and F.M. Lai, Departments of Medicine and Pathology, The Chinese University of Hong Kong, Hong Kong.* Glomerular deposition of HBV antigens is observed in chronic HBsAg carriers with different glomerulonephritides, yet the pathologic role of HBV remains uncertain. We examined the paraffin section of kidney biopsies from 40 chronic HBsAg carriers with membranous nephropathy (MGN), mesangiocapillary glomerulonephritis (MCGN), or IgA nephropathy (IgAN) for HBV DNA using *in situ* hybridization (ISH). Glomerular HBV antigens were present in all biopsies by immunofluorescence. HBsAg or HBcAg mRNA was studied in RNA extracted from frozen renal tissue using a two-step polymerase chain reaction (PCR) following reverse transcription (RT). HBeAg were detected in 79%, 50%, and 28% of sera from chronic HBsAg carriers with MGN, MCGN, and IgAN, respectively. HBcAg or HBsAg DNA was not easily detected with ISH alone, but was readily found in 31 biopsies (78%) following PCR. HBV DNA was detected mainly in the cytoplasm of the proximal tubular epithelia but not in glomerular cells. HBsAg and/or HBcAg mRNA were detected in 13 biopsies (33%). The PCR findings were further confirmed by: (a) Southern blot hybridization using a cloned HBV probe; and (b) absence of PCR product following treating RNA with RNase or omitting the RT. The detection of HBV DNA and RNA in kidney tissue indicates HBV viral replication in these HBV-related glomerulonephritides. The localization of HBV only in renal tubular epithelia but not in glomerular cells suggests these are circulating immune complex-mediated diseases. The development of a particular glomerular pathology (MGN, MCGN, or IgAN) in any individual HBsAg carrier is likely, in part, influenced by the HBeAg status in circulation.

Proteinuria in early aminonucleoside nephrosis (PAN) is associated with *in situ* glomerular reactive oxygen species (ROS) generation and lipid peroxidation (LPO). *T.J. Neale, P.F. Davis, N.S. Greenhill, B.M. Rüger, and D. Kerjaschki, Wellington School of Medicine NZ, and University of Vienna, Austria.* ROS are implicated in proteinuria production in PAN, a model of human minimal change GN. Up-regulation of respiratory burst cytochrome b558 mRNA in PAN glomeruli, and increased b558 protein expression in glomerular epithelial cells (GEC) 7 days after disease induction, suggested an intrinsic glomerular cell origin for ROS. Macrophages/glomerular cross-section (gcs) were similar in PAN and controls (1.25 ± 0.10 vs. 1.40 ± 0.10 macs/gcs; NS). Glomerular $[H_2O_2]$ *in vivo* was quantitated by catalase assay after aminotriazole administration (control 29.36 ± 2.54 SEM vs. PAN 14.05 ± 0.82 nM H_2O_2 consumed/min/mg protein; $P < 0.005$), and *ex vivo* by scopoletin assay (PAN 2.15 ± 0.52 SEM vs. control 0.29 ± 0.05 nmol H_2O_2 production/mg glomeruli; $P < 0.005$). H_2O_2 was localized by *ex vivo* cerium EM histochemistry to GBMs and GEC in PAN but was absent in controls. Malondialdehyde (MDA) was detected with antibody specific for MDA-lysine adducts in PAN glomerular sections and denuded GBMs by IF. Lipid peroxides were quantitated in glomeruli (PAN 209.4 ± 6.9 vs. control 148.0 ± 7.6 SEM nmol MDA/g glomeruli; $P < 0.005$) and on isolated GBMs (PAN 16.93 ± 0.33 vs. control 12.40 ± 0.02 SEM nmol MDA per mg protein; $P < 0.005$). Immunoblot with anti-MDA antibody on PAN glomerular extracts indicated that an ~220 kDa protein (shown to be type IV collagen derivatized in its NC-1 and triple helical domains), received MDA adducts. The functional role of LPO in PAN was proven by prior treatment with the

anti-LPO agent probucol (P), which markedly reduced proteinuria (P-treated 28 ± 6 vs. PAN 186 ± 8 mg protein per 24 hrs; $P < 0.01$), the IF signal for MDA and the of MDA concentration within glomeruli (41.0 ± 1.45 PAN vs. P & PAN 29 ± 0.85 ng MDA/mg protein; $P < 0.01$). Simvastatin pre-treatment (serum cholesterol reduction equivalent to P-pre-treated rats) did not inhibit proteinuria in PAN. ROS are generated *in situ* in PAN and LPO-induced MDA adduct formation on glomerular cells, and type IV collagen within GBMs is associated with production of proteinuria.

Expression of CD44 by mesangial cells in culture, in the THY 1.1 model and in human mesangial proliferative glomerulonephritis. *P. Roy-Chaudhury, T. Khong, J.H. Williams, N.E. Haites, B. Wu, and D.A. Power, Departments of Medicine and Therapeutics and Molecular and Cell Biology, University of Aberdeen, Scotland, United Kingdom, and The Immunology Research Centre, St. Vincent's Hospital, Melbourne, Australia.* Cytokines are important in the pathogenesis of mesangial proliferative glomerulonephritis (MPGN). Binding to proteoglycans in the extracellular matrix regulates cytokine function. Since the transmembrane molecule CD44 is a receptor for the proteoglycan hyaluronic acid and may itself possess glycosaminoglycan side chains, we studied its expression by mesangial cells in mesangial proliferative glomerulonephritis. Immunohistochemical staining for CD44 in the mesangium was markedly increased at 4 days after induction of the rat Thy 1.1 model of mesangial proliferative glomerulonephritis but declined over the next 5 days. This coincided with the peak of mesangial cell proliferation and macrophage infiltration assessed by staining for α-smooth muscle actin and proliferating cell nuclear antigen (PCNA) and the macrophage marker ED-1. Fifteen human renal biopsies with IgA nephropathy, however, showed no change in mesangial staining for CD44 with two anti-CD44 monoclonal antibodies. There was strong staining of infiltrating cells, cellular crescents and cells present within tubules, confirmed by staining of another 64 renal biopsies with a variety of diseases and compared with the pattern in 5 normal kidneys. Cultured human mesangial cells expressed CD44 strongly when assayed by immunohistochemistry, immunoprecipitation and Northern blot. Mesangial cells expressed the 85 kDa protein and mRNA species of 1.6, 2.2 and 5.0 kb typical of mesodermal cells. CD44, therefore, is another example of a protein strongly expressed by mesangial cells *in vitro* and weakly or not at all *in vivo*, but which is up-regulated in a disease model. It was not, however, up-regulated by mesangial cells in human mesangial proliferative glomerulonephritis.

Gene expression of TGF-β_1 and SPARC in the rat remnant kidney. *L.L. Wu, A. Cox, R. Gilbert, M. Cooper, and C.J. Roe, University of Melbourne, Department of Medicine, Heidelberg Hospital, Victoria, Australia.* Over production of cytokine transforming growth factor (TGF-β_1) has been found to promote matrix accumulation in several animal models of kidney disease. The molecular mechanism involving interactions between growth factors and extracellular matrix (ECM) remain unclear. Secreted protein, acidic and rich in cysteine (SPARC), participates in tissue repair by regulating the turnover of ECM and is itself regulated by TGF-β_1. This study evaluates the gene expression of TGF-β_1 and SPARC in a rat remnant kidney model following variable renal ablation. Male Sprague-Dawley rats weighing 200 to 250 g were randomly divided into 5/6th (FSN, $N = 30$) and 1/6th (OSN, $N = 30$) ablation. Ten rats from each group were killed at 4, 12 and 16 weeks post-surgery. Remnant kidney weight, systolic blood pressure, 24-hour urine protein and serum creatinine were measured. Histologic abnormalities were quantitated by point-counting. mRNA expression of TGF-β_1 and SPARC were evaluated by Northern analysis. FSN developed progressive renal failure, hypertension and proteinuria associated with marked tubulointerstitial fibrosis and glomerulosclerosis compared to OSN. There was a 2.0- ($P < 0.05$), 2.2- ($P < 0.05$) and 1.8 ($P < 0.01$)-fold increase in mRNA TGF-β_1 expression in FSN versus OSN at 4, 12 and 16 weeks, respectively. SPARC was also increased 1.8- ($P < 0.05$), 1.6- ($P < 0.05$) and 1.9 ($P < 0.01$)-fold, respectively, in FSN versus OSN. Interstitial fibrosis correlated with both TGF-β_1 and SPARC expression. We conclude that in 5/6th renal ablation there is persistent expression of TGF-β_1 and SPARC in association with progressive renal failure and interstitial fibrosis. Further investigation of the roles of TGF-β_1 and SPARC in progressive renal failure is warranted.

Advanced glycation is an important pathogenic process in experimental diabetic nephropathy. *T. Soulis, S. Sastra, V. Thallas, J. Berka, B. McWiliam, R.P. Murray-McIntosh, R. Gilbert, T.J. Neale, G. Jerums, and M.E. Cooper, Departments of Medicine, Heidelberg Repatriation/Austin and Wellington Hospitals, Australia and New Zealand.* Advanced glycated end products (AGEs) that accumulate as part of a non-enzymatically mediated reaction between glucose and proteins, are thought to play an important role in the development of diabetic nephropathy. Our own studies have shown an increase in AGEs, as measured by their specific fluorescence (Ex370, Em 440 nm), after 32 weeks in the streptozotocin diabetic kidney [Control (C) 6.5 ± 1.0, Diabetic (D) 25.4 ± 2.4 units/mg protein], which can be prevented by aminoguanidine, a phenyl hydrazine derivative [(DA) 7.7 ± 0.9 units/mg protein]. These findings were confirmed by measuring AGEs using a specific radioimmunoassay (C 2827 ± 883, D 7765 ± 492, DA 3549 ± 239 units/ng RNAase). A receptor for these AGEs known as RAGE has been recently cloned from bovine lung. The present study has explored the localization of both AGEs and RAGE in rat kidney. Formalin-fixed and paraffin embedded tissue sections were cut from normal Sprague-Dawley rats ($N = 9$), rehydrated, incubated in protein blocking agent for 20 minutes at room temperature, washed in PBS, and exposed to primary antibody (rabbit anti-AGE-KLH or anti-human recombinant RAGE) for 30 minutes at room temperature. Samples were washed again and incubated with biotinylated goat anti-rabbit IgG followed by peroxidase-conjugated avidin. Diaminobenzidine tetrachloride was used to reveal localization of peroxidase conjugates. There was widespread staining in lung parenchymal tissue for RAGE (positive control for RAGE) and AGEs. In the kidney, there was co-localization of AGEs and RAGE in collecting ducts and distal tubules. In addition, there was staining of the macula densa, afferent arterioles and the podocytes within the glomeruli. There was no significant staining of proximal tubules. There was also co-localization of both AGEs and RAGE in other tissues that are susceptible to diabetic vascular injury such as the retina and aorta. The co-localization of AGEs and their receptor in sites of diabetic microvascular injury may indicate a mechanism for the genesis of diabetic complications. Modulation of the receptor for advanced glycated end products or inhibition of AGE formation with aminoguanidine have the potential to influence the progression of diabetic nephropathy.

Heparin-binding epidermal growth factor (HB-EGF) mRNA expression in mesangial cells and the THY 1.1 model. *M. Polihronis, M. Pearse, B. Murphy, and D. Power, Immunology Research Centre, St. Vincent's Hospital, Fitzroy, Victoria, Australia.* HB-EGF is a one of several ligands for the epidermal growth factor receptor. It is a potent mitogen for smooth muscle cells, fibroblasts and keratinocytes but not endothelial cells, and is synthesized by smooth muscle cells and macrophages. To determine whether this cytokine is involved in the pathogenesis of mesangial proliferative glomerulonephritis, we have amplified a 510 bp cDNA fragment encoding mature rat HB-EGF by PCR and cloned it into pGEM-T. The fragment was completely sequenced on one strand and found to be identical to the published rat sequence. This fragment was labeled with ^{32}P and used to probe Northern blots of total RNA from rat mesangial cells. A 2.5 kb band was obtained in cells growing at confluence in medium containing 10% fetal calf serum. mRNA for HB-EGF was not detectable in serum-starved cells. Treatment of the cells with cycloheximide showed that HB-EGF is an immediate-early gene for these cells. Cytokines (PDGF-BB, EGF, TGF-α, TNF-α, TGF-β) also induced its synthesis but none was as effective as 10% serum. *In situ* hybridization studies were then performed using the non-radioactive digoxigenin system. mRNA transcripts were present in every cell of a population of cycling mesangial cells by *in situ* hybridization, suggesting that HB-EGF mRNA levels were not cell cycle dependent. In normal kidney, HB-EGF mRNA transcripts were present in occasional tubules by *in situ* hybridization and the glomeruli were negative. Within 30 minutes after induction of the Thy 1.1 model, however, there was expression within the glomerulus, Bowman's capsule and an increased number of tubules. Staining within the glomerulus was more marked at day 4 after disease induction but declined thereafter. These studies show that HB-EGF mRNA is produced by cultured mesangial cells and suggest that HB-EGF may be involved in the pathogenesis of the Thy 1.1 model of glomerulonephritis.

Local macrophage proliferation in glomerular crescent formation in experimental anti-GBM glomerulonephritis (GN) in the rat. *H.Y. Lan, D.J. Nikolic-Paterson, M. Mu, and R.C. Atkins. Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* We have previously demonstrated that macrophages are the major cell type within cellular

crescents in experimental anti-GBM GN and that macrophages play an important role in crescent formation and development. The present study addressed the issue of whether macrophage accumulation within cellular crescents was the result of cell recruitment or if local proliferation played a role. Macrophage proliferation was investigated in rat accelerated anti-GBM GN using a newly developed, sensitive double-immunostaining method in which microwave oven heating was used to retrieve cytoplasmic and nuclear antigens and to denature bound native antibodies/enzymes during the first round monoclonal antibody (mAb) staining. This treatment completely prevents antibody cross-reactivity during the second round of mAb staining. Macrophages were detected by cytoplasmic labeling with the ED1 mAb and proliferating cells were detected by nuclear staining with a mAb (PC10) against the proliferating cell nuclear antigen (PCNA). This system allowed clear identification of $ED1^+PCNA^+$ double positive cells. Groups of 4 rats were killed on days 14, 21 and 28 after injection of anti-GBM serum. All animals developed significant glomerular crescent formation (25–80%) and $ED1^+$ macrophages was the major cell type involved, accounting for 50–80% of total crescent cells. A dramatic finding was that 61 ± 3.2% of $ED1^+$ macrophages within crescents expressed PCNA, accounting for 73 ± 1.1% of total $PCNA^+$ cells within crescents. The local nature of macrophage proliferation within crescents was demonstrated by the complete lack of PCNA expression in blood monocytes. In addition, proliferating macrophages were invariably associated with areas of focal glomerular sclerosis and local Bowman's capsular rupture, suggesting that these cells play an important role in mediating severe local tissue injury. In conclusion, this is the first study to demonstrate that local macrophage proliferation is a major mechanism in macrophage accumulation during crescent formation and suggests that proliferating macrophages also participate in mediation of severe tissue injury.

Long-term follow-up of adults with reflux nephropathy. *Y.Y. Zhang and R.R. Bailey, Department of Nephrology, Christchurch Hospital, Christchurch, New Zealand.* To assess the long-term follow-up of patients with reflux nephropathy, we reviewed 294 patients over 15 years of age (females 235; Caucasians 288) who were on our departmental computer database on 28 Feb. 1994 and had not reached end-stage renal failure. The mean age of the patients at first presentation was 17.3 years (SD 14.4 years; range 3 weeks–72 years). One hundred and seventy-six of the patients had unilateral reflux nephropathy and 118 bilateral reflux nephropathy diagnosed on intravenous urography and/or DMSA scintigraphy. At initial presentation the majority of patients, particularly women, presented with a urinary tract infection, 25 (8.5%) had hypertension, 15 (5.1%) loin or abdominal pain, 14 (4.8%) proteinuria and six (2%) renal insufficiency. At the last follow-up (mean age 34.2 years; SD 13.7; range 15 to 81) 113 patients (38%) had hypertension or were on antihypertensive therapy. This was significantly more frequent in those with severe bilateral renal parenchymal scarring. Proteinuria was present in 92 (31%) patients; 40 of these had a creatinine clearance <70 ml/min. A total of 71 patients (24%) had a creatinine clearance <70 ml/min and 43 (15%) a urinary tract infection. A urinary calculus had occurred in 18 patients (2%). Hypertension, proteinuria and renal insufficiency were significantly more frequent in those with severe bilateral reflux nephropathy. We conclude that patients with reflux nephropathy, and particularly those who have proteinuria, hypertension or renal insufficiency, should be under long-term nephrological follow-up.

Thin basement membrane disease—clinical features compared with IgA nephropathy. *R. Auwardt, J. Savige, R. Sinclair, W. Heale, P. Miach, and D. Wilson, Renal Unit, Austin Hospital, Heidelberg, Victoria, Australia.* Thin basement membrane disease and IgA nephropathy are recognized as common causes of persistent hematuria. The full clinical spectrum and prognosis of thin membrane disease, however, requires further definition. This study aims to clarify this and compare the clinical features of the two conditions. Available medical records of 71 patients with a pathological diagnosis on electron microscopy of thin membrane nephropathy were examined. Similarly, medical records of 32 patients with a diagnosis of IgA nephropathy, made on the basis of renal biopsy histology and immunoflourescence, were also examined. The age range of the two groups of patients are similar: 10 to 68 years (median 34 years) for thin membrane disease and 5 to 61 years (median 31 years) for IgA nephropathy. However, thin membrane disease was significantly more common in females (84.5% female, 60 of 71) compared with IgA nephropathy (37.5%, 12 of 32). This may reflect a referral bias. There were statistically significant increases in the amount of hematuria and proteinuria found in patients with IgA nephropathy compared with thin basement membrane disease (*hematuria >100,000 RBCs/ml;* 83.4%, 26 of 31 vs. 44.6%, 25 of 56, $P < 0.001$; and *proteinuria >1 g;* 11.3%, 8 of 71 vs. 40.6%, 13 of 32, $P < 0.001$). In addition, at the time of biopsy patients with IgA nephropathy were more likely to have abnormal renal function (*Cr >0.11 mmol/liter,* 21.9%, 7 of 32 vs. 7.0%, 5 of 71, $P < 0.05$). At follow-up none of the 42 patients with thin membrane disease had progressed to a Cr >0.20 mmol/liter (followed for 6 months to 11 years, median 2.75 years) whereas 7 of 32 patients with IgA nephropathy had (followed for 7 months to 20 years, median 4.6 years, $P < 0.05$). Hypertension and macroscopic hematuria were also more common at presentation in IgA nephropathy but these differences were not statistically significant. In conclusion, the patients with IgA nephropathy were found to have more significant renal disease at the time of presentation and could progress to chronic renal failure. However, patients with thin basement membrane disease were characterized by microscopic or macroscopic hematuria with few other renal findings. Proteinuria occurs but is not in the nephrotic range and progressive renal impairment was not found. Although the long-term prognosis of thin membrane disease appears good, further long-term prospective follow-up studies are required.

Ocular abnormalities in thin basement membrane disease. *J.A. Savige, D. Colville, P. Branley, and D. Wilson, University Department of Medicine, Department of Ophthalmopathy, Renal Unit, Austin Hospital, and Box Hill Hospital, Melbourne, Victoria, Australia.* Thin basement membrane disease is a common condition that is characterized by diffuse thinning of the glomerular basement membrane on ultrastructural examination. A thinned glomerular basement membrane is also found in Alport syndrome, and retinal dots, corneal and lens opacities occur in this condition because many structural proteins are common to Bruch's, Descemet's and the glomerular basement membranes. We have examined the eyes of 16 patients with thin basement membrane disease, whose mean glomerular basement membrane thickness was less than 250 nm. The patients had a median age of 48 years (range 30 to 63); 7/16 (44%) had glomerular hematuria greater than 100,000 RBC/ml; 11/16 (69%) had proteinuria greater than 0.15 g/24 hr and 5/16 (31%) had an elevated BP. Three individuals (19%) had a positive family history. Vision was tested, and the eyes examined by slit lamp, biomicroscopic examination and direct ophthalmoscopy. Five patients (31%) had white corneal dots, one a corneal dystrophy (6%) and lens dots were present in 2 patients (13%). Retinal dots were present in 11/16 individuals (69%), the number varied from 2 to 40, and they were principally located in the perimacular area. There was no associated impairment of vision. There was no correlation between the number of dots, and degree of glomerular hematuria or proteinuria. These dots occur but much less often in patients with other renal diseases. We are not certain of their origin but they may be degenerate retinal epithelial cells. Retinal dots, and abnormalities of the cornea and lens are common in patients with thin basement membrane disease as well as in patients with Alport syndrome.

Ocular abnormalities in Alport syndrome. *J. Savige, D. Colville, D. Wilson, P. Miach, W. Heale, G. Thomas, J. Agar, and P. Kerr, University Department of Medicine, Ophthalmology Unit and Renal Unit, Austin Hospital, Box Hill Hospital, and Renal Unit, Geelong Hospital and Renal Unit, Monash Medical Centre, Melbourne, Australia.* The characteristic ocular abnormalities in X-linked Alport syndrome are anterior lenticonus, and a dot and fleck retinopathy. We report here the ophthalmic findings in patients with X-linked and autosomal Alport syndrome (6 patients from 5 families, and 2 unrelated patients, respectively). The eyes were studied with a slit lamp, biomicroscopic examination with a 90D lens, direct ophthalmoscopy and fundal photographs. In X-linked Alport syndrome, anterior lenticonus was not observed, but a "scissor's reflex" suggesting early lenticonus was present in one individual. Small white retinal dots were demonstrated in all patients with X-linked Alport syndrome, even in those individuals with normal renal function and hearing. Other abnormalities included corneal arcus, corneal dystrophy and dots, and lens opacities. The patients with autosomal Alport syndrome had no anterior lenticonus but retinal dots were present. We have observed these dots in other individuals with and without renal disease and we are not certain of their significance. Anterior lenticonus is uncommon in patients with Alport syndrome. A florid retinopathy is found in some patients, but

perifoveal retinal dots are common as are other abnormalities of the cornea and lens.

Significance of focal and segmental hyalinosis and sclerosis (FSHS) in IgA nephropathy. *D.K. Packham, H-D. Yan, T.D. Hewitson, K.M. Nicholls, P.S. Kincaid-Smith, and G.J. Becker, Department of Nephrology, Royal Melbourne Hospital, Victoria, Australia.* Between 1971 and 1991, 845 patients were diagnosed as having IgA glomerulonephritis on renal biopsy performed at the Royal Melbourne Hospital. These patients were followed for a mean period of 53 months post-biopsy (range 0 to 336 months). By the end of follow-up 147 (17%) of patients had developed chronic renal failure (Cr > 0.2 mmol/liter) or end-stage renal failure. Presenting creatinine >0.12 mmol/liter hypertension, nephrotic range proteinuria, age >40 years and male gender, all correlated strongly on univariate analysis with the development of chronic renal failure or kidney disease (all $P < 0.0001$). However, a number of patients developing chronic renal failure or end-stage renal failure already had renal impairment (creatinine >0.12 mmol/liter at presentation). A separate comparison was performed of patients presenting with creatinine <0.12 mmol/liter and either developing chronic renal failure or end-stage renal failure within 5 years of biopsy ($N = 18$) and those with creatinine still <0.12 mmol/liter after 5 years of follow-up ($N = 186$). Of the 18 patients who deteriorated 6 (35%) were nephrotic at presentation and 9 (56%) had focal hyalinosis and sclerosis on renal biopsy. This compared with 5 (3%) patients with nephrotic range proteinuria and 16 (10%) patients with focal hyalinosis and sclerosis among the 186 patients who did not deteriorate ($P < 0.0001$). Thus, in patients with normal renal function at presentation, the presence of nephrotic range proteinuria or focal hyalinosis and sclerosis are strong predictors of adverse clinical outcome.

Tubular proteinuria as a marker of interstitial fibrosis in glomerular and nonglomerular diseases. *W. Hongwei, S.J. Chadban, and R.S. Nanra, Nephrology Unit, John Hunter Hospital, Newcastle, NSW, Australia.* A prospective cohort study was undertaken in 147 patients with renal biopsies to define the association between interstitial fibrosis (IF) and proteinuria (UPr); mean age 46 years (SD 18), female:male ratio 1:1.3, and mean S_{Cr} 0.14 mmol/L (range 0.05–0.78). The biopsy diagnoses were: primary glomerulonephritis, 94; chronic interstitial nephritis, 11; diabetic nephropathy, 3; "normal', 6; and others, 29. IF and tubular atrophy (TA) were graded semiquantitatively (Grades 0–3), and significant ($P < 0.01$) ranked correlation coefficients were obtained between IF and S_{Cr} ($r = 0.66$), U_{Pr}/Cr ($r = 0.31$), albuminuria (U_{Alb})/Cr ($r = 0.35$) and β2-microblogulinuria ($U_{\beta 2m}$)/Cr ($r = 0.36$); similar results were obtained with TA. % Global glomerular sclerosis (GGS) correlated only with S_{Cr} ($r = 0.49, P < 0.01$). Forty of 147 biopsies obtained by graded random selection were morphometrically analyzed, and significant correlation coefficients were obtained between % interstitial volume (%IV) and $1/S_{Cr}$ ($r = -0.75$, $P = 0.000$), log U_{Pr}/Cr ($r = 0.57$, $P = 0.000$), log U_{Alb}/Cr ($r = 0.61$, $P = 0.000$), log $U_{\beta 2m}/Cr$ ($r = 0.56$, $P = 0.000$), log% fractional excretion $(FE)_{\beta 2m}$ ($r = 0.55$, $P < 0.001$) and log% FE_{Alb} ($r = 0.68$, $P = 0.000$). %GGS correlated only with $1/S_{Cr}$ ($r = -0.68$, $P = 0.000$) and log% FE_{Alb} ($r = 0.39$, $P = 0.022$). Multivariable regression analysis of U_{Pr} indices with morphometric indices gave significant results only between %IV and log$U_{\beta 2m}/Cr$ ($P = 0.048$), and log%$FE_{\beta 2m}$ ($P = 0.001$). $U_{\beta 2m}/Cr > 0.05$ mg/mmol occurred in 44% of cases, and gave a sensitivity of 50%, specificity of 91% in predicting Grades 2 and 3 IF. It is concluded that increased $U_{\beta 2m}$ excretion may be used as a marker of IF in renal biopsies, particularly in glomerulonephritis, and therefore, represents an adverse prognostic indicator.

Syndrome X and insulin resistance/hyperinsulinemia (IR/HI): The basis of increased susceptibility of transitional populations to renal disease (RD)? *W. Hoy, Menzies School of Health Research, Darwin NT, and The Lovelace Institutes, Albuquerque, New Mexico, USA.* Australian Aborigines, U.S. Blacks and Hispanics, American Indians, Mexican Americans, Europid Indians, and urban South African Blacks have rising rates of type 2 diabetes and cardiovascular disease, and high rates of both diabetic (D) and nondiabetic (ND) renal failure. This suggests a general susceptibility to RD; it is probably marked by microalbuminuria/albuminuria (MA/A), and a link to the metabolic/vascular syndrome through IR/HI is proposed. IR/HI is widespread in these groups. In part familial, it segregates with higher blood pressures (BP), dyslipidemia and cardiovascular risk, and is necessary but not sufficient for development of hyperglycemia. MA/A is also widespread in both ND and D. It clusters in families, marks the IR/HI and prediabetic states, increases with rising BP and glycemia, and predicts, not only overt nephropathy, but also cardiovascular mortality, in both D and ND. Susceptibility to RD clusters in certain D families, is marked by MA/A and associated with higher BPs; biopsies often lack specific D changes. ND RD clusters in families, MA/A marks the susceptibility, expression and progression are driven by BP profiles, and immunologic and ultrastructural features can vary among family members, and in individuals over time. Glomerulomegaly, often with mesangial expansion, is frequent in D and ND subjects alike, and perhaps is due to hyperperfusion or hypertrophy associated with IR/HI and related growth factors. Other features are sometimes superimposed (diabetic change, inflammation, immune deposits, etc.). The hypothesis is supported by clustering of both D and ND RD in certain families, strong family histories of D and ultimate development of D in many subjects with ND RD, and submergence over time of ND-ESRD diagnoses within the (presumed) D-ESRD category, as the epidemic of diabetes waxes in a population. RD susceptibility thus becomes part of Syndrome X and its vasculopathy, and amenable to the same interventions. RD expression, like the progression of renal failure, becomes an interactive process, relaxing constraints of current etiologic and morphologic categories.

Dyslipidemia and its relationship with parameters of coagulation and fibrinolysis in renal disease. *A.B. Irish, Oxford Renal Unit, The Churchill Hospital, Oxford, England, United Kingdom.* To define whether the dyslipidemia of chronic renal disease (CRD) and dialysis could contribute to increased cardiovascular risk via induction of a hypercoagulable state; lipids and lipoprotein(a) [Lp(a)], factor VII coagulant activity (fVIIc), fibrinogen (fib), plasminogen activator inhibitor-1 activity (PAIa) and Prothrombin Fragment 1+2 (F1+2) were measured in 134 patients with CRD and compared with 32 healthy controls (N). Results by mode of treatment [mean ± SD except F1+2 and Lp(a) = median] are shown below.

Mode *(N)*	HD (28)	CAPD (30)
Lp(a)	280	257
PAIa	8.0 ± 4.2	12.9 ± 7.4
Fib	339 ± 51	417 ± 88
fVIIc	167 ± 64	189 ± 48
F1+2	0.44	0.50

Mode *(N)*	CRD (76)	N (32)
Lp(a)	305	113[a]
PAIa	11.1 ± 6.7	11.4 ± 6.5
Fib	445 ± 187	268 ± 53[a]
fVIIc	176 ± 63	139 ± 39[a]
F1+2	0.55	0.30[a]

Lp(a) mg/dl, fVIIc as %std, fib in mg/dl, F1+2 nM and PAIa AU/ml.
[a] $P < 0.05$ by ANOVA between groups

Compared with N, all renal groups demonstrated increased fib, fVIIc and F1+2, consistent with a hypercoagulable state. All renal groups had elevated triglycerides (TG) and Lp(a), but PAIa was not significantly different from N. In all patients combined, fib correlated with LDL-cholesterol ($r = 0.45$, $P = 0.0001$), fVIIc and PAIa correlated with TG ($r = 0.30$, $P < 0.005$ and $r = 0.37$, $P < 0.001$). F1+2 correlated with age ($r_s = 0.41$, $P < 0.001$) and modestly with fib and VIIc ($r_s = 0.20$, $r_s = 0.19$, $P = 0.08$) but not with any lipid fraction or creatinine. Lp(a) correlated with LDL (0.28, $P < 0.05$) and inversely with albumin (−0.22, $P < 0.05$). Although the correlation of triglycerides with activation of factor VII and with PAIa, and the increase in F1+2 and fibrinogen in all groups suggests that the hypercoagulability of CRD may, in part, be a consequence of the dyslipidemia, prospective evaluation following lipid reduction is required to establish whether these associations are causal, or arise from a common response to metabolic perturbations of the uremic state.

Fatal calciphylaxis. *G.S. Kirkland, R.D. Sinclair, H. Rotstein, and B. Murphy, Departments of Nephrology and Dermatology, St. Vincents Hospital, Fitzroy, Victoria, Australia.* Calciphylaxis is a rare and often fatal condition

most frequently seen in dialysis patients. It has been defined as a condition of hypersensitivity with initial sensitization of the tissues and then subsequent challenge with an agent with resultant calcium deposition. We describe 2 patients with calciphylaxis in whom the sensitizing agent appears to be hyperparathyroidism and the challenging agent warfarin. The patients had similar characteristics: female; ages 47 and 57 years; a long course of chronic renal failure before commencing hemodialysis; on warfarin for aortic valve replacements for 1 and 2 years, respectively; normal protein C and protein S levels; severe hyperparathyroidism; calcium/phosphate products well controlled until the development of symptomatic calciphylaxis; treatment with steroids before diagnosis made; surgical parathyroidectomy and a fatal outcome from gastrointestinal hemorrhage. Both patients exhibited very painful panniculitis, livedo reticularis, ischemic ulcers with eschar formation and palbable cord-like vessels with overlying cutaneous necrosis. The latter has not been previously described in calciphylaxis. We believe that the sensitizing agent in these cases was hyperparathyroidism and the challenging agent may have been long-term warfarin treatment. The latter has not been associated with calciphylaxis previously, but has been associated with skin necrosis in patients with protein C and S deficiency. In summary, we suggest that dialysis patients with severe hyperparathyroidism starting on warfarin are at increased risk of fatal calciphylaxis.

Use of pamidronate in the treatment of hypercalcaemia associated with renal failure. *J. Kanellis, G. Mangos, J.A. Charlesworth, and B.A. Pussell, Department of Nephrology, Prince Henry Hospital, Sydney, New South Wales, Australia.* The safety and efficacy of pamidronate (a bisphosphonate) are well documented for the treatment of hypercalcemia of malignancy and for decreasing the activity and bone pain of Paget's disease. The only report of its use in renal failure has been in 10 patients on dialysis who developed hypercalcemia due to the use of calcium-based phosphate binders and vitamin D. We recently observed two patients with hypercalcemia and renal failure with Paget's disease and without evidence of malignancy or sarcoidosis. Hyperparathyroidism may have been a contributing factor in one case but both patients had normal levels of parathyroid hormone-related peptide. Serum calcium (Ca) decreased to normal levels in both patients following intravenous (i.v.) pamidronate. The first patient had widespread Paget's disease, significant renal impairment (creatinine 0.38 mmol/liter) and hypercalcemia (corrected serum Ca 3.02 mmol/liter; normal 2.10–2.55 mmol/liter) with marked hypercalciuria (24-hr urinary Ca 10.9 mmol; normal 2.5 to 7.5 mmol). He had a 15 year history of recurrent calculus formation and ureteric obstruction. Medications could not be implicated in the cause of the hypercalcemia and there was no history of immobilization. Following a single dose of 60 mg i.v. pamidronate, the serum Ca fell to 2.23 mmol/liter by day 7 and the urinary Ca fell to 3.3 mmol/24 hr. The serum Ca remained in the normal range one month after treatment. The second patient had Paget's disease localized to the ilium and had been on hemodialysis for 5 months. He had not received vitamin D supplements nor Ca containing phosphate binders. The corrected serum Ca was elevated for several months (range 2.60–2.94 mmol/liter) but rose further several weeks after commencing an aluminium-containing phosphate binder (range 3.34–3.86 mmol/liter). The parathyroid hormone level was not appropriately suppressed during this time (3.6 pmol/liter, normal 0.5–4.9 pmol/liter). Following a total dose of 90 mg i.v. pamidronate over 5 days (3 equal divided doses) the serum Ca fell to normal by day 10 and remained in the normal range two months after treatment. There were no detectable adverse effects following treatment in either patient. Intravenous pamidronate appears to be a safe and effective therapy for a variety of causes of hypercalcemia in patients with renal failure.

Partial parathyroid hormone (PTH) suppression by calcitriol in hypercalcemic renal transplant patients. *D.W. Johnson, A. Brown, H.D. McIntyre, and C.M. Hawley, Department of Nephrology, Princess Alexandra Hospital, Brisbane, Queensland, Australia.* Hypercalcemia due to persistent hyperparathyroidism post-renal transplantation is common and can engender significant bony morbidity. To assess the short-term efficacy and safety of calcitriol as a potential treatment, we studied ten stable renal transplant patients (3 males, 7 females) with good allograft function and persistent hypercalcemia and hyperparathyroidism for more than one year after transplantation. Each patient received a 1 μg oral dose of calcitriol followed by regular biochemical monitoring of serum and urine for one week. PTH fell significantly by 20% from 11.5 $\pm$ 2.7 pmol/liter pre-treatment to a nadir of 9.4 $\pm$ 2.1 pmol/liter at 48 hours ($P < 0.05$), and then returned towards baseline. Fractional excretion of calcium rose from 0.60 $\pm$ 0.14% at baseline to 0.90 $\pm$ 0.14% at 48 hours ($P < 0.05$). No significant changes were seen in serum corrected calcium, free calcium, phosphate or fractional excretion of phosphate throughout the study. We conclude that post-renal transplant hyperparathyroidism is neither fixed nor autonomous and can be partially suppressed by a single oral dose of calcitriol without exacerbating hypercalcemia. Longer-term studies of the role of oral calcitriol in post-transplant hypercalcemia are therefore justified.

Role of dual-energy X-ray absorptiometry (DEXA) bone densitometry in CAPD patients. *D.W. Johnson, R.J. Rigby, H.D. McIntyre, A. Brown, and J. Freeman, Department of Nephrology, Princess Alexandra Hospital, Brisbane, Queensland, Australia.* To assess the clinical utility of bone densitometry, we measured total body (TB), anteroposterior lumbar spine (APL), femoral neck (FN), Ward's Triangle (WT) and skull bone mineral densities (BMDs) using DEXA in 45 CAPD patients (19 males, 26 females). BMDs were then correlated with clinical, biochemical and radiographic indices of uremic osteodystrophy. BMDs were not significantly different from age- and sex-matched reference population data. Considerable regional variation of BMD Z-scores were noted between FN (-0.11 ± 0.23), WT (-0.11 ± 0.22) and APL (1.22 ± 0.04). APL Z-scores were significantly reduced in patients with prior fracture (-1.36 ± 1.07 vs. 0.89 ± 0.31), bone pain (-0.72 ± 1.08 vs. 1.01 ± 0.31) or steroid treatment (-0.62 ± 0.39 vs. 1.16 ± 0.35). Increased BMD Z-scores for APL (1.82 ± 0.57 vs. 0.38 ± 0.29, $P < 0.05$), FN (0.32 ± 0.36 vs. -0.38 ± 0.29, $P = 0.14$) and WT (0.45 ± 0.38 vs. -0.45 ± 0.26, $P < 0.05$) were found in patients with radiographic hyperparathyroid bone disease. Both APL BMD Z-scores and skull BMDs were weakly correlated with PTH ($r = 0.33$, $P < 0.05$ and $r = -0.33$, $P < 0.05$, respectively) and with CAPD duration ($r = 0.30$, $P < 0.05$ and $r = -0.30$, $P < 0.05$, respectively). Generally, however, TB and regional BMDs were poorly related to age, renal disease type, CAPD and renal failure durations, serum aluminium, calcium, phosphate, alkaline phosphatase, osteocalcin and PTH. We conclude that the prevalence of osteopenia is not increased in CAPD patients. Clinical and biochemical parameters do not reliably predict BMD measurements, but prior steroids and bone symptoms are major risk factors for important bone loss. Although DEXA can reliably detect osteopenia in different skeletal regions, its usefulness in detecting osteodystrophy is limited by confounding hyperparathyroid osteosclerosis.

Ultrasound assessment of joint involvement in dialysis related amyloidosis (DRA). *R. Ptaznik, M. Lanteri and J.K. Dawborn. Department of Radiology Austin Hospital and Renal Units, Fairfield and Austin Hospitals, Melbourne, Victoria, Australia.* DRA causes arthropathy, tendon thickening and damage, pathological fractures, carpal tunnel syndrome and "amyloid hand." To plan treatment strategies it is important to clarify the diagnosis and accurately define the structural changes in affected joints and the extent of disease. We studied 25 chronic hemodialysis patients with ultrasound to demonstrate changes in their shoulders, the most commonly affected joint, as well as the hips and knees. Thirteen patients demonstrated evidence of DRA in the shoulder, and symptoms related to the severity of the US changes. These included: altered echogenicity of the subscapularis and supraspinatus tendons (patchy hyper- and hypo-echoic regions) in 22 joints; hypoechoic material surrounding the biceps tendon in 19 joints; enhancement of cartilage reflection; punched-out erosions of the humerus with soft-tissue intrusion; thickening and loculation of the subacromial bursa and acromioclavicular joint, and thickening of the tendons. Rotator cuff tears were present in 11 shoulders, and were asymptomatic in 4, symptomatic in apparently normal tendons in 3 and symptomatic in association with apparent DRA in 4. Hip and knee symptomatology was not as pronounced, but thickening of the periarticular tissue of the hip was associated with evidence of amyloid deposition in the shoulder. DRA can be demonstrated by US examination of the texture and anatomy of the tissues of the shoulder joint and other affected joints. These changes correlate with symptomatology, enabling non-invasive diagnosis and assists in planning management and in study of the natural history of the condition.

Changes in the carpal tunnel and hand in dialysis related amyloidosis (DRA). *M. Lanteri, R. Ptaznik, and J.K. Dawborn. Renal Unit Fairfield Hospital, Fairfield and Department of Radiology Austin Hospital, Heidelberg,*

Victoria, Australia. Recurrent carpal tunnel syndrome (CTS) is a common manifestation of DRA in hemodialysis patients. They also develop a stiffness of the hands and fingers interfering with hand function. Amyloid deposition in the tissues of the wrist and hand is the main cause of these syndromes. We have used ultrasound to study the wrists and hands of 25 chronic dialysis patients and correlate the changes related to amyloid deposition with clinically relevant symptoms. Hypoechoic masses and erosions of the radial cortex, and/or enlargement of a bursa above the pronator quadratus muscle, were seen in 14 wrists, associated with CTS in 12 cases. In 6 other wrists with CTS these changes were not present, but there was thickening of the tissues of the carpus when compared with asymptomatic patients. The appearances suggest that amyloid is deposited deep in the carpus, and would not be accessible to routine CTR and perineural tissue sampling. Five patients with gross hand stiffness with limitation of both flexion and extension of the fingers displayed amyloid infiltration of the long flexor and extensor tendons and tendon sheaths in the hand explaining their disability. DRA causing CTS and "amyloid hand" can be simply and non-invasively demonstrated by ultrasound allowing study of the natural history of the condition.

ATP synthesis in exercising leg muscle of dialyzed and undialyzed uremic patients. *C.H. Thompson, A. Irish, G.J. Kemp, B. Rajagopalan, P. Styles, D.J. Taylor, and G.K. Radda, MRC Biochemical and Clinical Magnetic Resonance Unit, Oxford Radcliffe Hospital, Oxford, England, United Kingdom.* Fatigue in chronic renal failure (CRF) may be partly due to metabolic defects in skeletal muscle unrelated to any reduction in hemoglobin (Hb). We used ^{31}P magnetic resonance spectroscopy to study bioenergetics in gastrocnemius in CRF during exercise until fatigue and subsequent recovery. Dialyzed patients were compared with controls and with undialyzed patients with CRF. Changes in pH and the phosphocreatine (PCr) concentration allowed measurement of the effective mitochondrial capacity (Q_{max}) (calculated from the PCr recovery rate) and the rates of glycogenolytic (L) and oxidative (Q) ATP synthesis during exercise. Near infrared spectroscopy estimated end-exercise muscle oxygen supply.

Measurement (mean ± SEM)	Dialyzed $N = 13$	Undialyzed (creat. > 200) $N = 5$	Control $N = 33$
[Hb] *g/dl*	10.2 ± 0.4	10.8 ± 1.0	
3 min exercise			
−L *mM/min*	8 ± 2[ab]	1 ± 0.5	3 ± 1
−Q *mM/min*	14 ± 2[b]	19 ± 5	21 ± 2
Duration *min*	5 ± 1[ab]	10 ± 2	12 ± 1
Q_{max} *mM/min*	30 ± 3[ab]	49 ± 9	49 ± 3

[a] $P < 0.05$ cf undialyzed, [b] $P < 0.05$ cf control)

After exercise, dialysed patients have reduced muscle re-oxygenation rate as well as muscle blood flow. They show a significant reduction in effective mitochondrial capacity resulting in a compensatory increase in glycogenolytic ATP synthesis. These bioenergetic abnormalities did not correlate with exercise duration or with creatinine, [Hb] or re-oxygenation half-time. Compared to controls, there was no metabolic abnormality in undialyzed patients despite similar [Hb] to the dialyzed patients. Exercise duration was significantly reduced in dialyzed patients but there is no simple metabolic cause for this.

Acute bone edema in renal transplant recipients. *H.L. Pilmore, and R.J. Walker, Department of Nephrology, Dunedin Hospital, Dunedin, New Zealand.* Acute migratory bone pain occurring in the early months post-renal transplantation is unusual. We report two cases of acute severe bone pain 2–3 months post-living related kidney transplantation. The pain was predominantly in the weight bearing joints and apart from the pain, there were minimal clinical signs. Both recipients were on triple immunosuppressive therapy with normal graft function and no previous history of renal bone disease. Cyclosporine was stopped in one case with no alteration in symptoms. Plain X-rays were normal, and bone scans demonstrated increased tracer uptake in all affected joints but were not diagnostic. Bone alkaline phosphatase levels were markedly elevated. Magnetic resonance imaging (MRI) defined the lesions more clearly. There was decreased intensity in affected joints in T1 weighted images and enhanced brightness in T2 weighted and fat suppressed (STIR) images consistent with bone edema. MRI allowed the differentiation between migratory edema and the early development of avascular necrosis. In Case 1 the changes on MRI were poorly demarcated and serial scans demonstrated changes in bone edema; the bone pain resolved spontaneously over 3 months. Case 2 had serial changes on MRI demonstrated demarcated lesions in the femoral and tibial condyles of both knees highly suggestive of early avascular necrosis. Surgical intraosseous pressures (elevated) and intraosseous venography (diminished flow) and histology from decompression bone cores confirmed early avascular necrosis. The bone pain resolved over the next 3 months. These cases highlight the advantage of serial MRI in the diagnosis and management of acute bone pain in renal transplant recipients.

Recurrent pain in renal autotransplants for loin pain hematuria syndrome. *A. Parnham, A.J. Low, D. Perlman, P. Finch, and M. Thomas, Department of Nephrology, Urology and Anaesthetics, Royal Perth Hospital; and Perth Pain Management Centre, Perth, Australia.* More than 50 autotransplants have been performed worldwide for the loin pain hematuria syndrome, with an estimated recurrence rate of 10%. The local experience of autotransplantation for this condition was reviewed retrospectively. Over a two year period, 12 renal autotransplants, including one sequential bilateral procedure, were performed on 11 women. The median age was 42 years (29–48) and they had had intermittent loin pain for 2–28 years (median 7). Extensive urological investigations excluded structural causes for their symptoms. All were dependent upon strong narcotic analgesia at the time of operation. Patients were followed-up for 14–44 months (median 24). No patient had a deterioration in renal function. One diabetic patient with extensive vascular disease developed a renal artery thrombosis requiring transplant nephrectomy. One patient developed a "urinoma" requiring further surgery. Following surgery, all patients experienced complete relief of pain for at least two months. Three patients with four transplants have had no pain since the operation. Eight patients have had recurrent pain at the transplant site. In spite of this, five of these considered the operation worthwhile. Of the four patients with the most severe pain recurrence, three (75%) have a past or current history of depression, and did not have hematuria (vs. 14% of the remainder, $P = 0.09$, Fisher's exact test). These results demonstrate that renal autotransplantation is a nephron-sparing option with low morbidity for management of intractable nephralgia once conservative measures have been tried. Seventy-two percent of patients developed recurrent pain, usually of a lesser magnitude. A prior history of depression or absence of hematuria may be predictive of a poorer post-operative prognosis. A placebo response to surgery cannot be excluded.

Necrotizing fasciitis: A single center's experience. *H.L. Pilmore, R.J. Walker, J.A. Rietveld, P.G. Jones, J-C. Theis, D.A. Bowie, and A.G. Dempster, Departments of Nephrology, Orthopaedics, General Medicine, Intensive Care, and Pathology, Dunedin Hospital, Dunedin, New Zealand.* Necrotizing fasciitis is a rare but serious soft tissue infection with high morbidity and mortality. We wished to review our five year experience with this condition from 1989–1994. In addition, in light of recent interest in the association between necrotizing fasciitis and non-steroidal anti-inflammatory drugs (NSAID), we wished to determine the incidence of NSAID use in our necrotizing fasciitis patients. A review of all Dunedin Hospital cases of necrotizing fasciitis between January 1989 and June 1994 was undertaken. Subsequently, all specialists involved in treating the patients audited their notes, particularly regarding clinical presentation, complications, treatment; outcome and concomitant use of NSAIDs were also recorded. There has been a total of seven patients (four male) with a mortality rate of 43%. Survival was associated with early diagnosis, rapid and intensive medical and surgical intervention, and possibly the early use of hemofiltration. Five of the seven patients had ingested non-steroidal anti-inflammatory drugs prior to their presentation which may have potentiated the severity of the endotoxic shock. Necrotizing fasciitis remains a potentially lethal disease, but early management and aggressive treatment improve outcome. A high index of suspicion, avoidance of NSAIDs, and aggressive multidisciplinary team management of these patients offer the best chance of survival in necrotizing fasciitis.

Trial of subcutaneous octreotide administration in a patient with the hepatorenal syndrome (HRS). *M.A. Lonergan, M.J. Field, and B. Jones, Department of Medicine, University of Sydney, Concord Hospital, New South*

Wales, Australia. Impaired hepatic clearance of vasoactive peptides, including vasoactive intestinal peptide (VIP), which are released into the portal circulation, may contribute to the the altered sodium and water homeostasis in cirrhosis and hepatic failure. A beneficial response to subcutaneous octreotide, a somatostatin analogue, which blocks the release and action of a number of peptides including VIP, was reported in cirrhotic patients with ascites. In view of that report, we examined the effects of octreotide on renal function, sodium and water excretion and plasma levels of VIP, insulin and glucagon in a 44 year old female patient with alcoholic hepatitis who developed the hepatorenal syndrome. Octreotide was administered subcutaneously for 7 days on a reducing dose regimen. Following the commencement of octreotide a progressive fall in weight occurred. Plasma creatinine plateaued. Plasma sodium rose from 126 mmol/liter prior to octreotide peaking at 143 mmol/liter. Electrolyte-free water clearance fell as the dose of octreotide was reduced. Fractional excretion of sodium did not change, remaining very low (0.02% of filtered load). Absolute sodium excretion also remained low. Octreotide decreased the plasma levels of glucagon and insulin. However, these changes did not appear to correlate with the onset of renal effects. A small fall in plasma VIP levels also occurred. In summary, the use of octreotide in a single patient with the HRS was associated with some beneficial effects, specifically stabilization of the plasma creatinine, an increase in electrolyte-free water clearance and normalization of serum sodium. No increase in sodium excretion occurred. The alterations in the plasma levels of the regulatory peptides, insulin and glucagon, did not correlate with the rapid stabilization of GFR nor the onset of weight loss. These findings suggest a role for octreotide in the management of HRS, although the mechanisms are unclear.

The kidney in petrol sniffing. *M.G. Kirubakaran, Alice Springs Hospital, Alice Springs, NT, Australia.* Petrol sniffing is endemic among some of the central Australian Aboriginal communities who also have a high incidence of renal failure of unknown etiology. The toxic effects of sniffing petrol are usually attributed to the organic lead compound, tetraethyl, which is used as an anti-knocking agent in leaded petrol. While inorganic lead is well-known to be highly nephrotoxic, very little is known about the effect of organic lead on the kidneys. Acute toxicity due to excessive indulgence in petrol sniffing is usually associated with high blood lead levels, and a prospective study was undertaken to see if the kidneys were affected in such patients. A total of 48 patients admitted to Alice Springs Hospital with acute toxic effects of petrol sniffing were investigated systematically to determine the nature and extent of renal involvement, if any. The mean age of the patients studied was 19.8 years with a female:male ratio of 1:3.8. The duration of sniffing petrol ranged from 4 months to more than 10 years, with a mean of 17.2 months. The mean blood lead level was 3.23 μmol/liter (range 1.6–4.7; normal < 1.4). None of the patients studied had hypertension, anemia, hematuria or significant proteinuria, and their serum creatinine, urea, uric acid and electrolyte levels were all within normal limits. Fanconi syndrome was not detected in 4 patients tested. Autopsy was performed on 6 patients and their kidneys showed normal morphology with no evidence of changes due to lead toxicity. In a separate survey of 59 central Australian Aboriginal patients with established chronic renal failure (33 on maintenance hemodialysis, 14 awaiting dialysis and 12 renal transplant recipients), none gave a history of sniffing petrol in the past. This study does not show any evidence of acute or chronic renal damage as a result of sniffing petrol and the organic lead in leaded petrol does not appear to be nephrotoxic.

Low dose subcutaneous recombinant erythropoietin in children with chronic renal failure. *J.-R. Burke, Y. Mizusawa, M. Falk, P. Roy, P. Knight, A. Rosenberg, G. Kainer, E. Hodson, H.R. Powell, C. Jones, and R. Walker, Australian and New Zealand Paediatric Nephrology Association.* In a multicenter trial low dose subcutaneous (s.c.) recombinant human erythropoietin (rHuEPO) was evaluated in 22 children aged 4 months to 16 years, with anemia of chronic renal failure over a 12 month period. A commencing dosage of 50 μg/kg twice weekly was given until a target hemoglobin of 9–11 g/dl was achieved. The dosage was increased by 50 μ/kg per week, each four weeks, if the hemoglobin did not increase by 1 g/dl/month. When the target hemoglobin was achieved, the same weekly dosage was given as single injection. If the hemoglobin concentration exceeded 11 g/dl or fell below 9 g/dl the dosage was decreased or increased by 50 μ/kg weekly. After treatment the mean hemoglobin increased from 6.75 ± 0.76 to 9.6 ± 1.9 g/dl ($P < 0.001$), and hematocrit from 19.8 ± 2.4 to 29.3 ± 6.3 ($P < 0.001$). By four months the target hemoglobin was achieved in 19 patients on 50 μ/kg twice weekly and one patient on 75 μ/kg twice weekly. Two children with severe renal osteodystrophy failed to respond to 95 μ/kg and 150 μ/kg twice weekly. The maintenance weekly dose of rHuEPO in nine children over 4–12 months ranged between 45–125 μ/kg. There were no significant changes in the monthly mean values of serum iron, transferrin binding capacity, ferritin, parathormone, calcium, phosphate, alkaline phosphatase and creatinine. Full scale I.Q. in 11 children increased from 92 ± 16.1 to 97 ± 17 over the 12 month period (P = NS). No adverse effects were recorded. A starting dose of s.c. rHuEPO 50 μ/kg twice weekly is recommended as an effective and safe dosage for the majority of children with anemia of chronic renal failure.

Myeloma and renal failure. *A.F. Sharland, L. Snowdon, D.E. Joshua, J. Gibson, and D.J. Tiller, Departments of Nephrology and Haematology, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia.* Renal failure at presentation has been reported in up to 56% of myeloma patients and carries a poor prognosis. Limited data are available on the prognosis of those who are aggressively treated with dialysis. This study describes the myeloma population, diagnosed between 1986 and September 1993, and seen at our institution, with particular reference to renal function and outcome in patients who were dialyzed. Medical records were examined and evaluated if information was available about renal function at diagnosis, and there was follow-up for at least 12 months or until death. One hundred forty patients met the criteria for study. Their mean age was 62 years (29–87), 77 M and 63 F. Types of myeloma were; IgGK (56), IgGL (29), IgAL (16), IgAK (12), LLC (11), KLC (9), NS (6), and IgDL (1). Fifty patients had impaired renal function (serum creatinine >120 μmol/liter) at diagnosis, 14 were dialyzed, and 82% of patients with renal failure had stage III disease, compared with 43% of those with normal function. Median survival in the whole group was 34 months. Increased age, failure to attain plateau, and impaired renal function at diagnosis were independently associated with shortened survival. Median survival in the group with renal impairment was 22 months vs. 47 months in patients with normal renal function ($P < 0.01$). Median survival of dialyzed patients was also 22 months. Hypercalcemia and presence of Bence-Jones proteinuria were both significantly associated with renal failure at diagnosis ($P < 0.01$). Sixty percent of patients with light chain myeloma had renal impairment compared with 33% with other paraproteins and none of the 6 patients with non-secretory myeloma. Patients with renal failure at diagnosis have more advanced disease and a poorer prognosis. However, severe azotemia treated with dialysis confers no worse outcome than mild impairment, and this may continue to improve with new approaches, such as high dose therapy with stem cell rescue. Dialysis should be offered to patients with myeloma who require it.

Recovery of gastrointestinal function following renal transplantation in a patient with sclerosing peritonitis secondary to continuous ambulatory peritoneal dialysis (CAPD). *C.M. Hawley, R.J. Rigby, D.R. Wall, D.W. Johnson, S.B. Campbell, A.D. Griffin, and J.J.B. Petrie, Departments of Nephrology and Surgery, Princess Alexandra Hospital, Brisbane, Queensland, Australia.* We report the rapid and dramatic improvement in gastrointestinal function that occurred after a successful renal transplant in a 44 year old woman with sclerosing peritonitis secondary to CAPD. The patient had been on CAPD for 9 years and her sclerosing peritonitis was diagnosed after presenting with recurrent episodes of small bowel obstruction. CAT Scan had demonstrated severe peritoneal thickening and calcification, and ultrasound had shown rounded "nubbins" of tissue attached to the peritoneal surface. Colon transit time had shown markedly reduced colonic motility. The patient was converted to hemodialysis and trained for home total parenteral nutrition (TPN). After 9 months she received a cadaveric renal allograft. Immunosuppression was with cyclosporine, azathioprine and prednisolone. By 2 weeks post-transplant she was able to tolerate oral fluids, and by 12 weeks TPN was ceased as oral nutrition was adequate. She has continued to do well with no recurrence of abdominal symptoms (7 months post-transplant). We support the hypothesis of Junor et al that immunosuppression may be helpful in patients with sclerosing peritonitis, and propose that the anti-inflammatory effects of these drugs mediate the improvement in gut function.

Fat free mass (FFM) measurement in CAPD patients. *K.C. Wong, D.J. Borovnicar, P.G. Kerr, D.B. Stroud, B.J.G. Strauss, and R.C. Atkins, Monash Medical Centre, Clayton, Victoria, Australia.* As an index of nutritional

adequacy, FFM is not routinely assessed in dialysis patients because the tools for direct measurement of FFM are not readily available, and these techniques have not been validated in this group of patients. This study compared calculation of FFM by a reference four compartment model [FFMFCM = $TBWD_2O$ (total body water by Deuterium oxide dilution) + TBPrlVNAA (total body protein by *in vivo* neutron activation analysis) + TBBMDexa (total body bone mineral by dual energy x-ray absorptiometry) + glycogen (as 4.4% TBPr)], with values of FFM obtained from Dexa, BIA (bioelectric impedance), AN (anthropometry), TBK (total body potassium) and CK (creatinine kinetics), in a cohort of stable CAPD patients (6 M/12 F, age 33–77 years). FFMFCM showed a strong to moderate linear correlation with FFMDexa ($r = 0.93$, $P < 0.0001$), FFMBIA ($r = 0.89$, $P < 0.0001$), FFMAN ($r = 0.86$, $P < 0.0001$), FFMTBK ($r = 0.80$, $P < 0.0001$) and FFMCK ($r = 0.71$, $P < 0.001$), respectively. Intermethod comparison of group mean values of FFM showed no statistical difference between FFMFCM (46.9 ± 11 kg), FFMDexa (47.3 ± 12 kg) and FFMCK (44.3 ± 12 kg), respectively. Mean values of FFMBIA, FFMAN and FFMTBK were significantly different from that of FFMFCM ($P < 0.001$, $P < 0.05$ and $P < 0.005$, respectively). Measurement biases of +0.4 kg, +6.1 kg, +2.9 kg, −5.5 kg and −2.6 kg were observed for FFMDexa, FFMBIA, FFMAN, FFMTBK and FFMCK, respectively. Limits of agreement were the narrowest (+8.9 to −8.3 kg) for FFMDexa and greatest for FFMCK (+14.8 to −20.1 kg). As a group, CAPD patients had % hydration of FFM (76.6 ± 2.5%) greater than normal (published mean value for normal subjects ≈72%), but there was no gender difference in % hydration FFM (M 75.2 ± 1.3% vs. F 75.7 ± 3.1%). CAPD males had greater %FFM (of total body weight) (71.4 ± 6.8%) and %TBPr of FFM (18.4 ± 0.8%) than CAPD females (62.4 ± 10.8% and 17.4 ± 2.7, respectively) ($P < 0.01$, $P < 0.05$, respectively). Comparison of Z scores for FFM index (FFMI) [FFM (kg)/height(m)] and nitrogen index (NI) showed that FFMDexa was comparable to TBPr in categorizing nutritional status of individual CAPD patients. Our data suggest that FFM measurement is a suitable index of nutrition in CAPD patients and it can be accurately assessed by Dexa alone.

Predictors of technique failure in New Zealand CAPD patients (1986–1993). *J. Collins, G. Gamble, and J. Leary, for the NZ CAPD Registry, Renal Unit, Auckland Hospital, Auckland, New Zealand.* The New Zealand (NZ) CAPD registry collects comprehensive data on all NZ patients on CAPD after October 1986. Nine hundred thirty-three new patients commenced CAPD over this period. We performed multivariate stepwise logistic regression analysis to determine the predictors of technique failure (including death but excluding successful transplants) at 18 months and 3 years of treatment. Standardized odds ratios were calculated for each variable after adjustment for all other variables in the model.

Independent predictors of 18 month CAPD failure

	Standardized odds ratio	2P
Male gender	1.2	0.0104
Diabetic	1.3	0.0001
Early school leaver	1.2	0.0147
Treatment for hypertension (prior to dialysis)	1.1	0.0271
Years of past cigarette smoking	1.4	0.0001
Current cigarette smoking	1.2	0.0165
Previous dialysis experience	1.3	0.0004
Number of episodes of peritonitis	1.6	0.0001

The predictors of 3 year failure were similar to 18 month failure, except that current cigarette smoking and age when they left school were no longer significant. Variables included in the model but which failed to reach statistical significance were age at commencement of CAPD, tertiary education, race, independence in bag changing, an index of cardiovascular risk, marital status and visual impairment (all $P > 0.05$). We conclude that the number of episodes of peritonitis experienced by an individual is the strongest predictor of both 18 month and 3 year CAPD failure. Patients with two or more episodes of peritonitis in their first year are 60% more likely to fail CAPD within 18 months than those with one or no episodes of peritonitis. Previously, in univariate models race (Maori, Pacific Island vs. European) has been an important determinant of CAPD failure. In these analyses, after multivariate adjustment, race was not found to be important.

Time-related peritoneal function changes in long-term CAPD: The apex time. *J. Sabto, A. Slingeneyer, and B. Laroche, Clinique du Mas De Rocher, Castelnau le Lez, France.* Exposure to long-term CAPD may be accompanied by peritoneal morphological changes. Peritoneal function tests have not reflected these changes and have not predicted peritoneal membrane failure when it has occurred. The APEX time corresponds to the point of intersection of two equilibration curves: urea and glucose. The test was performed 133 times in 103 CAPD patients. The APEX time measured within the first month of CAPD was 58.79 ± 14.30 minutes; between 1 and 12 months after the start of CAPD 64.98 ± 20.30 minutes; between 12 and 36 months 65.09 ± 16.53 minutes, while that measured in patients on CAPD for longer than 36 months was 86.74 ± 31.72 minutes. These changes were statistically significant (analysis of variance f = 8.61 for df1 = 3, df2 = 129; $P < 0.005$). In a subgroup of 14 patients who had a first test in the first month of CAPD and at least one subsequent measure more than 3 months later, a rise in the APEX time was demonstrated (paired *t*-test $P < 0.05$). The APEX time combines an index of urea and glucose transport into one measurement. It does so at a time when transport rates are greater than those measured at 4 hours as is usual in the simplified Peritoneal Equilibration Test (PET). It is probably due to these two reasons that it has been brought to light the time-related peritoneal functional changes, and it is therefore likely to be useful in detecting and anticipating deterioration in peritoneal membrane function in long-term CAPD patients.

The elderly dialysis patient: Quality of life (QOL) and social activity. *H.R. Moody, C. Moody, E. Szabo, and C.M. Kjellstrand, Sir Charles Gairdner Hospital W.A. (Dept. of Renal Medicine); and University of Alberta Hospitals, Edmonton, AB, Canada.* Elderly patients may be denied the benefits of dialysis due to perceptions of poor QOL while receiving this therapy. We have performed an extensive survey of our dialysis and transplant population to ascertain QOL using the Karnovsky scale, perceived health, Campbell's and Bradburn's scales. In addition we assessed parameters of physical function and social activity. The patients were divided into young (16–39 years, $N = 20$), middle aged (40–59 years, $N = 23$), old (60–69 years, $N = 20$) and the very old (> 70 years, $N = 23$). While physical ability declined with increasing age (95% of those < 39 years were capable of all activities of daily living (ADL), only 63% of those > 70 years were capable of all ADL: young patients appreciated dialysis least, older patients the most. Seventy percent of those < 39 years perceived their health as worse than their peers while only 35% of those > 70 years felt this way ($P < 0.05$). Of the young, 10% perceived life to be totally miserable on dialysis, while no very old patient held to this belief. Approximately 20% of the young and very old were happy with their existence on dialysis and 70% of the very old said that life in general was quite satisfactory, while only 50% of the of the young said this. Participation in hobbies, outdoor activities, cleaning, laundry and cooking clearly changed beyond age 75. Approximately 55–85% of the young, middle-aged and old were involved in these activities, while only 25% of the very old participated. Elderly dialysis patients enjoy a satisfactory QOL, often exceeding that of the younger dialysis patient. While there appears to be a breakpoint in social activities at age 75 years and there is no doubt that physical activity declines with age, there is no justification to exclude the elderly from dialysis on the basis of poor QOL.

Sleep apnea and end-stage renal disease. *M. Hallett, S. Burden, D. Stewart, and P. Farrell, University of New South Wales, ResCare Ltd, New South Wales, Australia.* Previous studies have indicated that there may be an association between sleep apnea (SA) and end-stage renal disease (ESRD), regardless of modality of therapy. Full sleep studies were therefore done on a number of dialysis patients to obtain further information on prevalence of SA in this group. Fifteen patients were randomly selected from 4 Sydney metropolitan dialysis centers, [9 on hemodialysis (HD) and 6 on continuous ambulatory peritoneal dialysis (CAPD)]. All subjects underwent full overnight polysomnography at a sleep disorders center, with measurement of sleep and breathing variables. A respiratory disturbance index (RDI) was calculated for both NREM and REM, and a combined RDI for all subjects. Four out of 6 CAPD patients

and 8 out of 9 HD patients had evidence of sleep apnea (combined RDI > 5/hr). One out of 6 CAPD and 6 out of 9 HD patients had evidence of moderate to severe sleep apnea with an RDI > 15/hr. Apneas in all patients were exclusively obstructive in nature. The other finding of interest was that there was a high incidence of periodic leg movements during sleep in this patient group, with 2 out of 6 CAPD and 4 out of 9 HD patients exhibiting this disorder. This initial study was extended to cover more patients in both the U.S. (F. Finkelstein, New Haven) and Australia (J. Mahony/R. Caterson, Sydney). In this case, OSA was assessed by either Edentrace II (New Haven patients) and ResCare's Clinical System coupled with pulse oximetry (Sydney patients). In the New Haven sample of 15 CAPD patients, 7 patients (47%) had an RDI > 5 while 4 had more severe OSA with an RDI > 15 (27%). However, in the Sydney sample of 21 dialysis patients, 9/11 (82%) HD patients and 7/10 (70%) CAPD patients had an RDI > 15. The combined data show that for all patients at the 3 centers, 72.5% had an RDI > 5 and 53% had an RDI > 15. These results confirm the high incidence of sleep disordered breathing in patients with ESRD on dialysis therapy. The link between OSA and cardiovascular and cerebrovascular disorders is now recognized. As these are the most common causes of death in the dialysis population, treatment of sleep apnea by nasal CPAP should be of considerable benefit to these patients.

Varying concentration and delivery of dialysate bicarbonate: Effect on phosphate removal. *E. Yuill, D. Chesher, and D.C.H. Harris, Departments of Renal Medicine and Clinical Chemistry, Westmead Hospital, New South Wales, Australia.* High bicarbonate (HB) dialysis may be used to reduce osteodystrophy and other complications of acidosis, but may impair phosphate (P_i) clearance by increasing cell uptake of P_i. It has been proposed that P_i clearance may be improved by bicarbonate modelling (BM), with early low dialysate HCO_3 (to increase clearance) and late high HCO_3 (to correct acidosis). To test the utility of BM and HB in low-flux dialysis, 12 stable chronic haemodialysis patients received standard bicarbonate (SB, dialysate HCO_3: 28–30 mmol/liter), HB (dialysate HCO_3: 40 mmol/liter) or BM (dialysate HCO_3: increasing exponentially from 28 to 40; 35 mmol/liter at 3 hrs) for 4 weeks each in a randomized double-crossover design. Oral bicarbonate supplements were used during HB and BM. Nine patients completed the trial. During week 4 of each treatment dialysis kinetics of P_i, urea, HCO_3, K^+ and Ca^{2+}, plasma lipids (triglycerides, cholesterol, HDL cholesterol) and patient tolerance were assessed during both of the second and third dialysis sessions. Pre- and post-dialysis serum HCO_3 was significantly (all $P < 0.003$) lower in SB (20.2 ± 1.0, 23.8 ± 0.7 mmol/liter, respectively, $\mu \pm$ SE) than HB (26.1 ± 1.1, 29.7 ± 1.4) and BM (25.4 ± 0.6, 27.3 ± 0.6). There was no difference among SB, HB and BM in plasma P_i at any time point, in 2 hours post-dialysis P_i rebound (0.33 ± 0.04, 0.34 ± 0.06, 0.29 ± 0.03) or in clearance of P_i from blood (such as at one hour, 87.9 ± 3.0, 91.3 ± 1.7, 92.7 ± 2.4 ml/min). P_i clearance tended to rise during SB, but fall with HB and BM. There were no changes in plasma lipids. Other biochemical data, including dialysate P_i appearance, are yet to be analyzed. Patient tolerance was equal with each treatment. In summary, acidosis can be controlled well by low-flux dialysis using HB, without any measurable reduction in phosphate clearance. BM has no demonstrable advantage over HB.

Kinetic modelling in assessment of adequacy of hemodialysis (HD). *C.A. Pollock, F.Y-P. Zhu, W. Ayass, R.J. Caterson, J.F. Mahony, D.A. Waugh, S.D. Roger, and L.S. Ibels, Department of Renal Medicine, Royal North Shore Hospital, New South Wales, Australia.* In order to assess the value of urea kinetic modelling and nutritional markers in HD, 76 patients (32 M, 44 F; aged 55 ± 3.1 vs. 63.1 ± 2.1 years, respectively; $P < 0.01$) underwent dialytic and urinary measurements of urea generation rate, from which Kt/V and protein catabolic rate (PCR) were determined. Hospital admission rates, infectious episodes and access complications were recorded. At the commencement of the study, these patients had been on dialysis for 41.9 ± 5.7 (mean ± SEM) months, with 19 patients undergoing assessment within 1 month of the commencement of dialysis. During the 12 month study period, 47 patients (61.8%) required admission, spending 7.9 ± 1.4 days in the hospital (range 2–146 days): 29 patients (38.1%) had at least one infectious complication requiring antibiotic therapy; 18 patients (23.6%) developed access problems needing intervention; and 27 patients (35.5%) had symptomatic peripheral vascular or ischemic heart disease. Multivariant analyses demonstrated that female patients had more frequent ($P < 0.01$) and longer ($P < 0.05$) hospital admissions, but no difference was observed in Kt/V or PCR when compared to males. PCR correlated inversely with hospital admission rates ($P < 0.01$), total days spent in hospital ($P < 0.05$) and infectious complications ($P < 0.05$). Infectious complications further correlated with time on dialysis ($P < 0.005$) and the presence of diabetes ($P < 0.005$), and inversely with total body nitrogen ($P < 0.05$). Diabetes was associated with increased ($P < 0.05$) and longer ($P < 0.001$) hospitalization, increased access problems ($P < 0.001$), but no difference in Kt/V, PCR, TBN or vascular pathology was observed. Vascular pathology was associated with more frequent hospitalization ($P < 0.05$), a lower PCR ($P < 0.01$) and increased access complications ($P < 0.05$), but not with Kt/V. In conclusion, urea kinetic modelling provides predictive value with respect to dialysis related morbidity. Measurement of PCR is of more prognostic value than Kt/V in the HD population.

Factors determining Kt/V and protein catabolic rate (PCR) in hemodialysis (HD). *C.A. Pollock, F.Y-P. Zhu, W. Ayass, R.J. Caterson, J.F. Mahony, D.A. Waugh, S.D. Roger, and L.S. Ibels, Department of Renal Medicine, Royal North Shore Hospital, New South Wales, Australia.* The stability of markers of measurements derived from urea kinetic modelling in HD is unknown, and factors which modify them are generally assumed rather than measured. The stability of measurement of Kt/V and PCR was therefore assessed in 12 hemodialysis patients by repeated determinations one week apart. This demonstrated excellent reproducibilty with coefficients of variation of 6.8% and 9.1%, respectively. In 45 patients (15 M, 30 F) Kt/V and PCR were assessed on 2 occasions, 6 months apart. Time on dialysis, blood flow rate and dialyzer type were recorded. Kt/V at the commencement of the study was 1.23 ± 0.04 and at the conclusion of the study 1.41 ± 0.06 ($P < 0.005$). PCR was 1.06 ± 0.04 and 1.31 ± 0.05 g/kg/day, respectively ($P < 0.001$). During the study 30 patients had an increase in Kt/V of 0.33 ± 0.04 associated with an increase in PCR of 0.24 ± 0.06 g/kg/day, 7 patients had a decrease in Kt/V of 0.33 ± 0.01 with a decrease in PCR of 0.002 ± 0.1, and 8 patients had stable Kt/V measurements. Of the 30 patients who increased their Kt/V, 11 increased their hours of dialysis alone, 2 increased the dialyzer size alone, 1 increased the blood flow rate alone, 6 patients increased all three parameters, 5 altered 2 of the three parameters, while 5 had no discernible change in their dialysis regimen. An increase in Kt/V correlated with an increase in time on dialysis ($P < 0.02$), but not with a change in either blood flow rate, which in these patients averaged 58 ± 12 ml/min ($P = 0.49$), or type of dialyzer ($P = 0.56$). A close correlation existed between Kt/V and PCR ($P < 0.001$). An increased PCR was observed with increased time on dialysis ($P < 0.05$) and increased dialyzer size ($P < 0.05$), but not with an increased blood flow rate. In the 7 patients whose Kt/V decreased, PCR decreased in 3 and was unchanged in 3. Of these 7 patients, access problems were identified in 4 patients, suggesting this should be suspected if unexplained decreases in Kt/V occur. In the 1 patient with a disproportionate increase in PCR sepsis was recognized. We conclude that both Kt/V and PCR are reproducible measures. Both Kt/V and PCR may be increased by more time on dialysis, whereas PCR may be further increased by an increased dialyzer size.

Specific detection of membrane inserted C5b-9(m) complexes in human renal biopsies. *B.F. Murphy, M. Polihronis, D. Power, and D. Machet, Departments of Nephrology and Clinical Immunology, St. Vincent's Hospital, Fitzroy, Victoria, Australia.* We have recently characterized a murine monoclonal antibody (254 Mab) which identifies a neoepitope on complement C9 which is revealed only when C9 is in the membrane bound (that is: lytic) terminal C5b-9(m) complex. This Mab is distinguished from all other anti-C5b-9 neoepitope antibodies in that it does not react with the soluble, non-lytic, SC5b-9 terminal complex. Both SC5b-9 and C5b-9(m) are present in glomerular immune deposits and at other sites in pathological kidneys, and it has not been previously possible to distinguish immunohistologically between the pathogenic C5b-9(m) complex and the "inactive" SC5b-9 complex. The 254 Mab has now enabled, for the first time, a distinction between these 2 complexes to that made in human tissue. We have performed a prospective immunofluorescence study of 200 percutaneous renal biopsies comparing the distribution of C5b-9(m) with that of other complement components and immunoglobulins. The following antibodies were used: 254 MAb to detect C5b-9(m), conventional anti-C9 neoepitope Mab to detect total C5b-9, anti-clusterin and anti-vitronectin Mabs to detect SC5b-9, and the routine polyclonal

antisera to detect immunoglobulin and early complement components (C1q, C3, IgG, IgA, IgM). Polyclonal antisera were FITC-conjugated and used directly, Mabs were used in indirect immunofluorescence with a secondary antibody [FITC-conjugated anti-mouse Ig (DAKO)]. C5b-9(m) was invariably detected as part of total C5b-9 in glomerular immune deposits and in most extraglomerular sites (vessels and tubular basement membrane). There was no C5b-9 detected with either antibody in histologically normal glomeruli. C5b-9(m) was also found together with immunoglobulin in a number of immune glomerular lesions where early complement components (C3, C1q) and SC5b-9 components were not detected. In particular, in IgA nephropathy, 5/19 biopsies showed this pattern. This suggests that in these biopsies only C5b-9(m) and not SC5b-9 was present. Finally, three cases of non-IgA diffuse mesangial proliferative GN showed C5b-9(m) deposition in the absence of antibody and other complement components, suggesting that direct activation of C5b-9(m) without antibody may occur in mesangial cells. These data confirm that membrane insertion of C5b-9 is an invariable feature of glomerular complement deposition and that the 254 Mab is a highly sensitive reagent for the detection of glomerular complement.

Tamm-Horsfall protein (THP) in rat anti-gbm glomerulonephritis (GN). I. Potential nephritogenic autoantigen. *H.Y. Lan, S. Qing, D.J. Nikolic-Paterson, and R.C. Atkins, Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* Tubulointerstitial leukocytic infiltration is a common feature of most forms of human and experimental GN and correlates better with the degree of renal function impairment than glomerular lesions. We hypothesize that this tubulointerstitial inflammation is, at least partially, an autoimmune response to intrinsic kidney antigens which are released as neo-antigens following initial renal damage. The nephritogenic potential of THP, a glycoprotein produced exclusively by tubular epithelial cells, was investigated in a study of rat accelerated anti-GBM GN. Disease was induced by injection of rabbit anti-GBM serum into rats primed with rabbit IgG and killed at various times over a 28 day time course. In normal rats, no circulating anti-THP was detectable by ELISA and no skin DTH response to purified THP was evident. In rat anti-GBM disease, significant levels of serum anti-THP antibodies were detected on day 7 which increased further to day 28. A marked skin DTH response to THP antigen was seen on day 7 which increased in severity up to day 28—indeed the reaction was similar in intensity to the DTH response to rabbit IgG (immunizing antigen). Double immunohistochemistry staining to localize THP and leukocyte subsets found that from day 14 onwards there was a 3–4-fold greater number of T cells and macrophages in contact with THP positive tubules compared to that around THP negative tubules. This was also the time of onset of severe tubulointerstitial damage and renal function impairment. In conclusion, an autoimmune response to THP was generated during rat anti-GBM disease as demonstrated by specific antibody production and skin DTH reaction. Furthermore, the association of leukocyte infiltration with THP positive tubules suggests an active role for the THP immune response in mediating renal injury in this model.

Infiltration of TH1 subsets into kidneys in Heymann's nephritis. *M.J. Penny, R. Boyd, A. Spinelli, and B.M. Hall, Department of Medicine, Liverpool Hospital, Liverpool, New South Wales, Australia.* Th1 cells are principally involved with delayed-type hypersensitivity (DTH) and cytotoxicity, and are characterized by expression of IL-2, IFN-γ, and TNF-β. Th2 cells are required for B cell maturation and Ig isotype switching and produce IL-4, IL-5, IL-6, IL-10, and IL-13. We examined the cytokine profiles in active Heymann's nephritis (HN), a disease model of membranous GN in which antibody and complement are thought to be mediators of injury. HN was induced in 5 male Lewis rats by subcutaneous injection of renal tubular antigen (Fx1A) in CFA, and boosted at two weeks with Fx1A in IFA. The control group (Controls) comprised 5 male Lewis rats which received the same induction protocol, without Fx1A. Disease in the HN group was demonstrated at 8 weeks. Proteinuria in the HN group was 206 ± 16 mg/100 g body weight/day (X ± SD) compared to 6 ± 3 mg/100 g body weight/day in Controls, $P < 0.001$. Mean auto-Ig titers (compared to normal serum titer corrected to 1.0) were 37.6 ± 14.0 in the HN group, and 1.6 ± 0.3 in Controls, $P = 0.002$. In renal biopsies glomerular Ig deposition was present in the HN group but absent in Controls, and immunoperoxidase cytochemistry of lymphocyte subsets in renal cortex (cells/HPF, X ± SD of 10 HPF) demonstrated:

Target	Control	HN	P
TCR	6.0 ± 1.7	24.6 ± 9.7	= 0.011
CD4	48.3 ± 8.0	97.6 ± 20.6	= 0.006
CD8	7.9 ± 1.3	11.3 ± 0.2	= 0.007
NK	1.5 ± 0.3	2.3 ± 1.5	= 0.003

mRMA extracted from renal cortex was assayed by a semiquantitative RT PCR:

TH cell	Cytokine	Control	HN
TH1	IFN-γ	±	+++
	IL-2	+	+++
	TNF-β/LT	±	+++
TH2	IL-4	±	+
	IL-10	−	+

This study identified increased numbers of T cells (CD4+ and CD8+) in Heymann's nephritis, with evidence of activation of TH1 subsets. This suggests DTH or cytolytic T cell activation may play a role in the mediation of this disease.

Mycophenolate mofetil prevents the induction of active Heymann's nephritis. *M.J. Penny, R. Boyd, A. Spinelli, and B.M. Hall, Department of Medicine, Liverpool Hospital, Liverpool, New South Wales, Australia.* This study examined the effectiveness of Mycophenolate mofetil (Mm) in suppression of active Heymann's Nephritis (HN) in rats. HN was induced in male Lewis rats by immunization with Fx1A in CFA, and boosted at 2 weeks with Fx1A in IFA. A control HN group was compared to a group given Mm for 4 weeks from the time of induction of HN (30 mg/kg/day, p.o.) and a normal group immunized only with adjuvant. HN activity was monitored by urine protein excretion and autoantibodies at 4, 6, 8 and 12 weeks, and renal biopsies at 4, 8, and 12 weeks. Biopsies were examined for Ig and C deposition. Renal cortical leukocyte infiltrates were examined using indirect immunoperoxidase staining with mAbs to cell markers, and the expression of cytokines by infiltrating cells was examined using RT PCR. In the Mm group proteinuria was not significantly increased when compared to the normal group (<10 mg/100 g body weight/day) at 6, 8 and 12 weeks but significantly less than the untreated HN group (>200 mg/100 g body weight/day, $P < 0.001$). Auto-Ig titers were suppressed to baseline levels during the four weeks of Mm therapy, but at 6, 8 and 12 weeks were significantly elevated compared to normals, $P < 0.011$, and not significantly different to the untreated HN group. There was marked glomerular Ig deposition in the Mm group which was only slightly less than the untreated HN group at 8 weeks and absent in the normal group. Significantly increased CD4+, CD8+, NK and monocyte/macrophage numbers were identified in HN controls compared to the Mm treated and normal control groups, $P < 0.016$ (there being no significant difference between Mm treated and normal control groups). RT PCR of cytokine mRNA in renal cortex demonstrated activation of antigen presenting cells/macrophages (TNF-α) and Th1 cells (IFN-γ, IL-2, TNF-β) in HN controls compared to the Mm treated and normal control groups. No increase in Th2 activity (IL-4, IL-10) was demonstrated. In conclusion, Mm prevented proteinuria and leukocyte infiltrates to 12 weeks, with suppression of Th1 and APC cytokine mRNA. Mm did inhibit auto-Ab titers and glomerular Ig and C deposits during the four weeks of therapy, but these appeared following cessation of Mm. This study suggests Mycophenolate mofetil may have a role in the treatment of glomerulonephritis.

Biased TCR Vγ gene usage by infiltrating $\gamma\delta$ T cells in IgAN. *J.F. Knight, G. Ng, G. Zhang, H-L. Wu, A.R. Clarkson, A.J. Woodroffe, L.P. Roy, and M.C. Falk, Renal Research Laboratories, The Children's Hospital, Camperdown, New South Wales, and Renal Unit, Royal Adelaide Hospital, Adelaide, SA, Australia.* Infiltration of the kidney by $\gamma\delta$ T cells is associated with progression to renal failure in IgA nephropathy (IgAN). It is yet to be determined whether these cells are recruited from peripheral blood as part of a non-specific inflammatory response, or whether they represent a selective subset of circulating $\gamma\delta$ T cells, infiltrating and proliferating in response to a particular antigen or antigens. To address this question, a T cell receptor (TCR) repertoire analysis was performed on blood and renal

tissue. mRNA was extracted from renal biopsies from 7 adults with IgAN who had been shown to have a significant γδ T cell infiltrate. rtPCR was performed with primers specific for the four TCR Vγ and six TCR Vδ gene subfamilies, as described. A kidney with pyelonephritis served as a positive control and the unaffected pole of a kidney removed for Wilms' tumor as a negative control. Because a biased repertoire in the kidney might simply reflect changes in peripheral blood, γδTCR repertoire was also assessed in blood from 12 IgAN patients (including four of the seven patients whose biopsies were examined) and from 12 healthy adults. PCR product was dot-blotted, hybridized with P^{32}-labelled oligonucleotide probes specific for TCR Cγ and Cδ, respectively and densitometry performed on an autoradiograph. TCR Vγ and Vδ repertoire in peripheral blood did not differ between IgAN patients and controls. TCR Vδ repertoire in the IgAN biopsies also did not vary from that seen in peripheral blood in patients and in healthy controls—all six families were represented in typical proportions. TCR Vγ repertoire was characterized by the marked absence of Vγ1, which was undetectable in five of the seven IgAN biopsies, although it was expressed by at least 20% of peripheral blood γδ T cells in all controls and IgAN patients, and 30% of γδ cells in the pyelonephritic kidney. In the four IgAN patients on whom both blood and tissue were available, TCR Vγ1 was 24–61% of blood γδTCR repertoire but only 0–1% of the renal infiltrate. These data suggest that the γδ T cells seen in IgAN biopsies are a selective subset of those circulating in peripheral blood, yet they do not appear to be a clonal population. The striking absence of Vγ1 requires further examination in a larger, prospective series, with a detailed sequence analysis of TCR gene usage.

P-selectin directs autologous antibody induced injury in the skin (passive Arthus reaction) but not in the glomerulus. *L. Santos, P.G. Tipping, X.R. Huang, M.C. Berndt, and S.R. Holdsworth, Centre for Inflammatory Disease, Monash University Department of Medicine, Monash Medical Centre, Clayton, and Baker Medical Research Institute, Prahran, Victoria, Australia.* Binding of autologous antibody to antigen presented in the skin and the glomerulus initiates local tissue injury. In the skin, this is known as the Arthus reaction and is characterized by complement dependent neutrophil accumulation. Rats given autologous rat anti-sheep globulin i.v. (10 mg/150 g body weight) develop skin swelling (2.85 ± 0.20 mm) and neutrophil accumulation 4 hours after intradermal challenge with sheep globulin (170 μg; passive Arthus reaction). This response was associated with local up-regulation of P-selectin on endothelial cells in intradermal vessels. Pretreatment with a functionally inhibitory rabbit anti-P-selectin antibody, 1 hour prior to induction of passive Arthus, prevented up-regulation of endothelial P-selectin, neutrophil accummulation and skin swelling (2.20 ± 0.04 mm, normal 1.94 ± 0.04 mm). These parameters were unaffected by pretreatment with non-immune rabbit Ig. Glomerular binding of passively administered autologous antibody to the same planted antigen (a subnephritogenic dose of sheep anti-rat GBM globulin) results in proteinuria (49 ± 9.5 mg/24 hr, normal 1.9 ± 0.5 mg/24 hr) and proliferative glomerulonephritis in rats and is also associated with up-regulation of P-selectin on glomerular endothelial cells. This form of glomerular injury is the theorectical equivalent of the dermal passive Arthus reaction but was associated with a predominant macrophage rather than neutrophil influx. The role of P-selectin in this passive autologous antibody initiated glomerular injury was assessed by *in vivo* inhibition using an identical dose of anti-P-selectin antibody to that employed to inhibit passive dermal Arthus. However, unlike dermal Arthus, glomerular injury was unaffected by inhibition of P-selectin (proteinuria 51 ± 9.5 mg/24 hr), suggesting that glomerular macrophage infiltration is P-selectin independent. Thus autologous antibody binding to antigen presented in the skin and glomerulus results in recruitment of a different profile of inflammatory cells dependent on different cell adhesion molecules.